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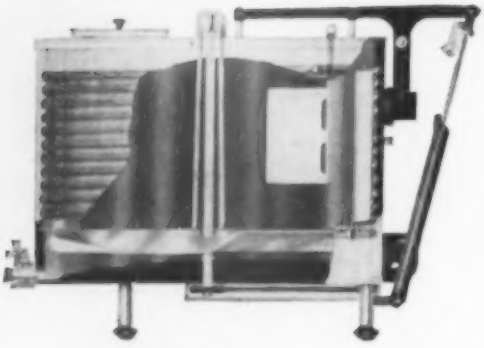

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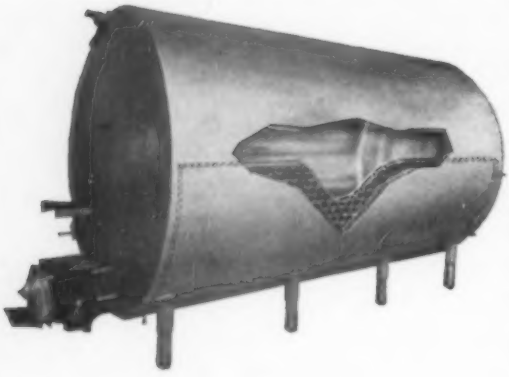

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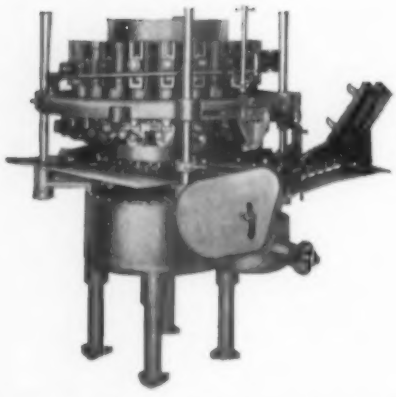

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The Storage Life of Frozen Orange Concentrate from the Standpoint of the Consumer

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(Manuscript received November 12, 1951)

A number of questions have arisen regarding the keeping quality of frozen orange concentrate after it has been thawed in preparation for serving. The necessity for storing the thawed concentrate may occur when serving a large number of people or in the periodic administration of small quantities such as is practiced in experimental and infant feeding. Judging from inquiries received, it would appear that many consumers are uncertain as to whether the frozen concentrate should be handled in the same manner as fresh juice or as the (single strength) canned juice. The present investigations were inaugurated for the purpose of obtaining information which might serve to answer some of the consumer's questions.

In the past, most of the investigators have either concerned themselves with the keeping quality of fresh orange juice or have reported on the effects of various temperatures on the unopened cans of frozen orange concentrate but there appears to be little information available on the keeping quality of frozen orange concentrate after it has been thawed and prepared for serving.

Rohrer and Treadwell *(5)*, working with Texas oranges, reported an average loss of 3.9% ascorbic acid in fresh orange juice which was held at room temperature for 24 hours. When stored at 40° F. (4.4° C.) the loss of ascorbic acid during 24, 48, and 72 hours was 4.3, 10.8, and 18.9%, respectively. When the (single-strength) orange juice was diluted with tap water and held at 40° F. for 24 hours the loss of ascorbic acid was 4.9% compared to 3.3% in the undiluted juice.

Moore, Atkins, Wiederhold, and MacDowell *(3)* conducted similar studies with Florida oranges. The freshly extracted juice was placed in covered and uncovered glass quart jars and stored at room temperature and in the cold room (40° F.). These investigators reported that after 3 days in the cold room both covered and uncovered juices were considered good in flavor and were found to have retained 98% of the original ascorbic acid. After a week in the cold room the juices had retained 96% of the ascorbic acid and the flavor was still considered good.

At room temperature the juices could be held only 2 days. The flavor was considered good at the end of this time. At the end of 3 days, fermentation had begun. It was found, however, that 98% of the ascorbic acid had been retained even though the juice was fermented. The investigators concluded that storage of orange juice in the home is limited by loss of palatability and beginning of fermentation, rather than by appreciable ascorbic acid losses.

Experiments on shipping fresh orange juice across continental United States were reported by the U. S. Department of Agriculture *(1)* in 1949. In these tests especial care was exerted in expressing the juice and the product was rapidly cooled to 30-32° F. (—1-0° C.). Carlots of this fresh orange juice, packed in 1-gallon cans and refrigerated with ice and salt, were successfully shipped from Los Angeles, California, to New York City. It was reported that orange juice prepared by this method will retain its flavor and vitamin C content for 2 weeks or longer under refrigeration. Even after 3 weeks of refrigerated storage the product was considered superior to heat-processed canned orange juice.

A preliminary report on storage changes in frozen concentrated citrus juices was released by Moore, Huggart, and Hill *(4)* in 1950. Experimental packs of frozen orange and grapefruit concentrates were held for 6 months at —8°, 10°, 20°, 32°, and 40° F. (—22°, —12°, —7°, 0°, and 4.4° C.). The results showed no appreciable decrease in ascorbic acid in any of the samples during the period of storage. Clarification and varying degrees of gelation occurred in all samples stored at 20°, 32°, and 40° F. The rate of clarification was greater than that of gelation at the above temperatures. Gelation and clarification occurred to some extent in some of the samples stored at 10° F. and in the Duncan grapefruit concentrate which was stored at —8° F. A greater degree of gelation and clarification was observed in the concentrates prepared from seedy varieties (Pineapple orange and Duncan grapefruit). The trend in gelation and clarification was followed by slight changes in flavor. A reduction in total microbiological count was observed to occur in samples stored at all temperatures, the rate of reduction being more rapid at the higher temperatures.

DuBois and Kew *(2)* reported on the effect of holding frozen orange concentrate at 75°, 45°, 25°, 20°, 15°, 10°, 5°, 0°, —8°, and —20° F. After 11 months at 0° F. or lower no significant changes were noted in cloud retention or viscosity of the juice, and there was no etching of the can. At 5° F. the change in cloud retention was slow but definite, and changes in the appearance of the can were noticeable in 110 days. At progressively higher temperatures changes occurred progressively more quickly, according to these investigators. Stability (well-distributed pulp and cloud) could be attained by maintaining the product at a sufficiently low temperature. The authors state that it is likely that the effects of storage at elevated temperatures are cumulative and irreversible.

As the storage period progressed there was a notable reduction in the percent of living microorganisms in the concentrate at all temperatures below 25° F.

A taste panel of 20 persons failed to discriminate between portions of the samples stored at —20° F. and those held at the various higher temperatures until

changes in physical appearance were well advanced.

Vitamin C losses were stated to be low even at the higher temperatures although details were not to be reported until later.

MATERIALS AND METHODS

The 6-ounce cans of frozen orange concentrate were purchased from local retail stores. The cans were opened at the laboratory, the contents thawed and thoroughly mixed. The concentrate was then divided into 2 lots. One was kept in the concentrated form. The other was reconstituted according to directions on the can. This consisted of mixing 3 parts of water with 1 of the concentrate. Unless otherwise stated, tap water was used for reconstituting the concentrate. The 2 lots were further sub-divided and samples of both concentrate and reconstituted orange juice were stored at 32°, 41°, and 77° F. (0°, 5°, and 25° C.). All samples were kept in glass quart jars with glass tops.

When samples of the concentrated juice were removed from storage for periodic observations, the juice was reconstituted before testing. This was done both for taste tests and for chemical analyses. Experimental errors were considerably reduced by employment of this technique.

A total of 9 different experiments were performed in this series of investigations, selecting 2 different commercial brands of frozen orange concentrate. At the beginning of these investigations the samples were stored in duplicate. When the results began to show slight though definite trends it was decided to increase the number of replicate samples to eliminate the possibility of errors resulting from manipulation. In the last 5 experiments each lot consisted of triplicate or quadruplicate samples. Thus a typical experiment would consist of 4 jars of concentrate and 4 jars of reconstituted juice at each of the 3 temperatures. The results of only 2 experiments, with their statistical significance, are reported here because they are typical of the others.

Ascorbic acid was determined as follows. First 5 ml. of the reconstituted juice were combined with 5 ml. of 3% metaphosphoric acid and the mixture was titrated with 2,6-dichlorobenzenoneindophenol. The results were expressed as mg. per 100 ml. of reconstituted juice.

It will be noted that the ascorbic acid in the original sample is reported for both the concentrate and the reconstituted sample. That is, the samples were prepared for storage and then analyzed. This procedure was followed for the purpose of compensating for any errors of manipulation involved in making the dilutions.

RESULTS

Samples of orange concentrate stored at 77° F. (25° C). for 24 hours usually developed a certain amount of "aged" flavor. This was true whether the juice was held as the concentrate or whether it was reconstituted and then stored. The juice was not always unpalatable but so much of the original flavor and aroma was lost that the quality was considered questionable. On the average, approximately 95% of the ascorbic acid was retained during the 24 hours at 77° F. (25° C.).

Orange concentrate held at 77° F. (25° C.) for 48 hours was definitely fermented. No difference in flavor was noted between the concentrated and reconstituted lots. The quantity of dye (2,6-dichlorobenzenoneindophenol) reduced by the juice was greater after 48 hours of storage than after 24 hours. This was interpreted to mean that increased reduction of the dye was caused by interfering substances and was not an indication that ascorbic acid had increased during storage. It is possible that some of the intermediate products of alcoholic fermentation may have reduced the dye. In fact some of these interfering substances may have been present at the end of 24 hours at 77° F. (25° C.), even before the final products of fermentation appeared. If so, then the results of ascorbic acid determinations may not be entirely reliable for the 24 hour lots at 77° F. (25° C.). Because of this and because of the question regarding the flavor, the storage at 77° F. (25° C.) was discontinued.

The results obtained with storage of the juice at 32° F. (0° C.) and 41° F. (5° C.) are presented in Table 1.

It will be seen that when the frozen orange concentrate was thawed and stored at 32° F. (0° C.) for 4 days it retained 95% of its original ascorbic acid. When stored in the reconstituted condition a similar amount of ascorbic acid was retained. (The slight differences between the two lots are not significant). During 8 days of storage at 32° F. (0° C.) a little more ascorbic acid was lost but the percent retention was still high (91-92.5%). There was no significant difference between the lots stored as concentrate or in reconstituted condition in so far as retention of ascorbic acid is concerned.

As would be expected, a 41° F. (5° C.) storage of the thawed concentrate was not as effective in retaining ascorbic acid as was 32° F. (0° C.) storage. However, the amount of ascorbic acid retained after 4 days of storage was still relatively high (92.3-92.7%). There was no significant difference between the ascorbic acid content of the concentrate and that of the reconstituted juice after 4 days at 41° F. (5° C.). After 8 days of storage at 41° F. (5° C.) there appeared a marked difference in the ascorbic acid content of the 2 lots. The percent retained was 91.2 in the concentrate and 85.6 in the reconstituted juice. The difference is statistically significant.

It appeared from these results that some oxidizing agent might be present in the tap water which is capable of causing oxidation of ascorbic acid. To test this theory, experiments were set up in which samples were reconstituted with both distilled and tap water. The

TABLE 1

Ascorbic acid content of frozen orange concentrate after 4 and 8 days of storage at 32° F. (0° C.) and 41° F. (5° C.) stored as the concentrate and in the reconstituted condition

Storage temperature	Ascorbic acid content									
	Original sample		After 4 days of storage				After 8 days of storage			
	Concentrate	Reconstituted	Concentrate		Reconstituted		Concentrate		Reconstituted	
	mg. per 100 ml.	mg. per 100 ml.	mg. per 100 ml.	Percent of original	mg. per 100 ml.	Percent of original	mg. per 100 ml.	Percent of original	mg. per 100 ml.	Percent of original
32° F. (0° C.)	58.2	57.7	55.2	95.0	56.0	96.9	53.7	92.5	52.6	91.0
41° F. (5° C.)	57.9	57.4	53.7	92.7	53.0	92.3	52.8	91.2	49.2	85.6

samples were stored at 41° F. (5° C.) only. These results appear in Table 2.

In so far as retention of ascorbic acid is concerned, the results obtained in this test were better than those of the previous one, 98.0 to 99.6% retention after 4 days and 94.5 to 99.3 after 8 days. In the original samples the lots reconstituted with distilled water were significantly higher in ascorbic acid than were those which were reconstituted with tap water. There was no significant difference between the concentrate and the sample which was reconstituted with tap water. Since the concentrate was reconstituted with tap water before being analyzed, these last results merely indicate that the degree of experimental error was not significant. Differences between the distilled-water and tap-water lots were again observed after both 4 and 8 days of storage at 41° F. (5° C.). In other words, when reconstituted with distilled water, the juice retained significantly higher quantities of ascorbic acid than if reconstituted with tap water.

TABLE 2

Ascorbic acid ontent of frozen orange concentrate after 4 and 8 days of storage at 41° F. (5° C.) using tap water and distilled water as reconstituting agents

Manner in which stored	Ascorbic acid content				
	Original sample	After 4 days of storage		After 8 days of storage	
	mg. per 100 ml.	mg. per 100 ml.	Percent of original	mg. per 100 ml.	Percent of original
Concentrate	39.4	38.6	99.6	36.6	94.5
Reconstituted with tap water	38.8	38.0	98.0	37.0	95.6
Reconstituted with distilled water	41.0	40.7	99.4	40.7	99.3

The results are interesting when considered from the standpoint of the amount of ascorbic acid lost by the different lots during storage for 8 days. (The loss during 4 days of storage is not statistically significant). There was a significant loss of ascorbic acid when the samples were stored for 8 days at 41° F. (5° C.) both as the concentrate and when reconstituted with tap water. The samples which were reconstituted with distilled water and stored for 8 days at 41° F. (5° C.), showed no significant loss of ascorbic acid at the end of this period.

FLAVOR

The flavor of the juices was tested by a panel of 10 individuals selected at random. These tasters were instructed to think in terms of fresh orange juice, frozen concentrate, and canned (single strength) juice. They were asked to decide whether a particular sample was (a) better than, (b) as good as, or (c) not as good as one of the 3 above-mentioned standard types of orange juice. These categories were given numerical values so that the evaluations of all the tasters could be averaged. The results of the taste tests were as follows. The thawed juice stored at 32° F. (0° C.) for 8 days was considered just as good in flavor as frozen orange concentrate served directly from the can. None of the tasters discriminated between concentrate and reconstituted juice which had been stored at 32° F. (0° C.). The samples which were stored at 41° F. (5° C.) were not considered as good as those stored at 32° F. (0° C.). In general the juice which was held at 41° F. (5° C.) for 8 days was rated as being better than canned juice but not quite as good as the original frozen concentrate. The concentrate after 8 days at 41° F. (5° C.) was slightly better than the reconstituted juice at this temperature.

The limit of the storage life of the thawed orange concentrate is not necessarily 8 days at 32° F. (0° C.). Preliminary tests showed that even after 10 or 12 days at 32° F. (0° C.) the juice showed no serious loss in palatability.

DISCUSSION

Although the preparation of frozen orange concentrate for serving is not as time consuming as is the case with fresh orange juice, it is nevertheless often necessary for the concentrate to be held for some time prior to its consumption. This applies not only to the individual family but also to institutions like schools, hospitals, and restaurants. Other investigators have previously reported that fresh orange juice may be kept in the refrigerator for a reasonable length of time without serious loss of ascorbic acid and without significant effect on flavor. The results now being reported indicate that frozen orange concentrate appears very much like fresh orange juice in its tolerance for brief periods of storage. The data are similar to those reported for fresh orange juice by Moore, Atkins, Wiederhold, and MacDowell *(3)*. That is, the length of storage is limited more by loss of palatability than by loss of ascorbic acid.

The superiority of distilled water over tap water in preserving the ascorbic acid in the juice is at least of academic interest. It is realized that it would not be practicable to substitute distilled water for tap water in commercial processing of any type of food. However, the data suggest an approach for additional investigations. It would be interesting to determine if the storage life of canned foods would be affected by the use of distilled water in the processing. If so, certain harmless but undesirable impurities might be removed from tap water without involving the expense of distillation. Ion-exchange resins have been employed for producing water with a high degree of purity and they might be of use at least in an experimental exploration of this field.

SUMMARY

Frozen orange concentrate was thawed and stored in glass containers at 77° F. (25° C.), 41° F. (5° C.) and 32° F. (0° C.) for various periods of time.

Although fermentation of the juice held at 77° F. (25° C.) was not recognizable until 48 hours, the flavor after 24 hours of storage was not always satisfactory so that storage at 77° F. (25° C.) was discontinued.

The orange concentrate was held at 41° F. (5° C.) for 4 days without serious loss of ascorbic acid and without impairment of flavor. Similar results were obtained after 8 day storage at 32° F. (0° C.).

Within the above limits of time the juice kept as well in the concentrated form as when reconstituted with tap water.

When distilled water was employed for reconstituting the concentrate a higher percentage of ascorbic acid was retained during storage. Although these results were statistically significant, the actual amount of ascorbic

acid involved was not large enough to make the use of distilled water practicable.

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Relation of Ketchup Color to Tomato Color as Determined by the Hunter Instrument[a]

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(Manuscript received June 27, 1951

The Hunter Color-Difference Meter has been used to study tomato and finished ketchup color in a full production operation for a ketchup factory. The color measurements made on heated extracted samples of whole tomatoes are correlated with the color measurements made on the finished ketchup. The significance of the data is discussed.

Interest in the objective measurement of tomato and tomato product color is evidenced by several recent publications *(1, 3, 4, 5, 6)*, and work undertaken by several of the state agricultural experiment stations and supported by the Production and Marketing Administration. The use and advantages of the Hunter Color Difference Meter in grading tomatoes for processing and in grading the processed products have been adequately described elsewhere *(2 3, 4, 5, 6)*.

The main interest in undertaking the present study was the desire to relate directly and objectively raw tomato color with the color of the processed product made therefrom, in this instance ketchup. This study represents the sampling of tomatoes and ketchup from the full production operation of an Ohio ketchup factory. The results indicate that differences in raw tomato color are reflected in changes in ketchup color to a surprising degree. In addition, the work shows that these differences can be measured accurately and expressed numerically.

Some data were collected in an evaluation of the present visual method of grading raw tomatoes by U. S. standards for juice grade tomatoes as compared with the Hunter color measurement of pulp prepared from graded tomatoes.

EXPERIMENTAL METHODS

The Hunter Color Difference Meter which measures color directly by reflectance was used for the color measurement *(2)*. The instrument was equipped with an R_d scale rather than an L scale. The instrument measures three tomato color factors: lightness or darkness (R_d), redness (a), and yellowness (b). Increasing a and b values indicate increasing redness and yellowness, respectively. An increasing R_d value indicates increasing lightness. The measurements were made with small area illumination and a small aperture.

Standardization. The instrument was standardized with a red porcelain enamel panel specially prepared and described by Younkin *(5, 6)* to match tomato color as closely as possible. The instrument is most accurate when the circuits are initially balanced with a color standard similar to that of the sample to be measured. The instrument was restandardized prior to each series of samples and showed insignificant, if any, drift after the initial warm-up.

Viewing cells. The sample viewing cells were made from 6 cm. lengths of standard Pyrex glass tubing of 64 mm. inside diameter, cemented to 3-inch square pieces of plate glass with Sauereisen "Insa-lute Adhesive Cement, No. 1 Paste," an acid-proof glass cement manufactured by the Sauereisen Cements Company, Pittsburgh, Pennsylvania. The base plate of the cells was prepared from ⅛-inch thick "Crystallex" plate glass (Pittsburgh Plate Glass Company). This particular glass was compared with cells prepared from spectacle and optical crown glass both with ketchup samples and spectrophotometrically. Although the optical crown glass (3/32 inch) showed a slightly higher transmission in the red, the differences in readings on the Hunter instrument between ketchup samples of different color were as great or greater for the "Crystallex" as for the optical glasses. The square base makes centering the cell on the instrument easier than when a round base is used.

Sample preparation. All color measurements were made on tomato pulp because of the uniformity attained. For each sample 8 to 10 pounds of tomatoes were used. The tomatoes were washed (but not trimmed), quartered, and placed in a small steam jacketed kettle (2 gallons). The sample was stirred as necessary to avoid scorching, but not sufficiently to macerate before a true "hot break" was attained. After heating to 190° F. (87.5° C.) as rapidly as possible, the heated tomatoes were pulped in a Model F "Sep-Ro-Siv" fitted with a screen with circular perforations 0.027 inch in diameter, operating at 144 r.p.m. Using a "hot break" and this size screen was sufficient to prevent rapid settling of the tomato solids after pulping. While the "hot break" does affect tomato color, the conditions of sample preparation were kept constant, and were comparable to one of the common methods for tomato pulp preparation. Using the "hot break" it was not necessary to deaerate the sample. After pulping, the sample was well mixed, approximately 1 pint was taken and immediately cooled in a stainless steel beaker in an ice water bath. After cooling to room temperature, the color was measured on the Hunter instrument and the 3 values for R_d, a, and b were determined.

Hue index calculation. As a means of simplifying the relation and presentation of data, Kramer's *(3)* hue value formula

[a] Presented at the Eleventh Annual Meeting of the IFT, New York, N. Y., June 19, 1951.

$(\frac{3b-15}{a})$ has been used in combining the Hunter *a* (red) and *b* (yellow) values into 1 numerical figure indicative of the overall hue or color value. We prefer to call this calculated result the color "Index" to avoid confusion with the Munsell method of color description which uses hue and value for 2 different color attributes. The calculation is such that the lower the color index number, the better is the color. In general, as tomato color improves, the *a* or red value increases as the *b* or yellow value decreases. This will be illustrated as the data are presented.

Lightness factor. *(R_d):* Data from the present study indicate that as the color improves the R_d or lightness value decreases indicating a darker color. As shown by others *(3, 4, 6)*, this lightness factor *(R_d)* does not contribute appreciably to tomato color, but hue is the basic factor. The two factors seem to parallel each other. As the color index decreases indicating better color, the R_d value usually decreases.

EXPERIMENTAL RESULTS

Relation of tomato to ketchup color. The effect of variations in color of raw tomato stock on the color of finished ketchup was studied in the full production operation of a northestern Ohio ketchup factory. Representative samples of tomatoes entering the factory were obtained by taking random samples (8-10 pounds) from each operating rig as the fruit was being transferred from the washer to the inspection rig. For example, if 4 rigs were operating, then 4 separate samples were taken at the same time. The samples were then heated, pulped, and the color measured on the Hunter. The average of these samples was then considered representative of the color of the tomatoes entering the factory at the time of sampling. This average color was then compared with the color of a ketchup sample taken immediately after filling at an interval subsequent to the raw fruit sampling estimated to equal the time required for processing from raw fruit to finished ketchup. Such comparisons were made throughout the 1950 tomato season with the color of approximately 500 samples being determined.

The results have been summarized on a weekly basis and the average weekly values as well as the ranges for the calculated Color Index numbers are represented in Figure 1. As the season progressed, it was realized that the ketchup color was running uniformly good throughout the season in spite of the cold and wet weather. Because of this limited range of ketchup color, there was

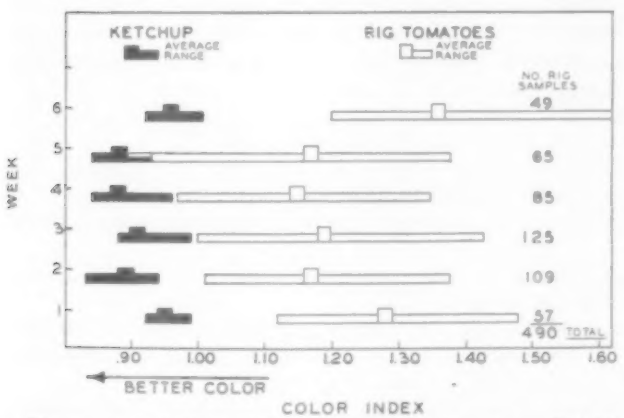

Figure 1. Relation of tomato to ketchup color. Weekly averages and ranges of color encountered throughout the 1950 tomato season. Rig tomatoes sampled after washing and before trimming.

some question as to the relation which might be expected between tomato and ketchup color. It is evident from Figure 1 that a very good relationship was obtained. The correlation coefficient between the Color Index for raw tomato color and finished ketchup color is 0.697, which is considered to be definitely significant. Further significance can be placed on the data because of the rather limited range of ketchup color encountered. No inferior ketchup color is represented in these data.

While the range for rig tomato color by the Hunter instrument appears rather wide, no information was obtained as to the actual color grade or distribution by the PMA (Production and Marketing Administration, U.S.D.A.) method of the tomatoes in the rigs at the time of sampling. Therefore, this relation between tomato and ketchup color cannot further be related to grade color with the present data. However, frequency distribution curves for the sets of weekly data show a normal distribution indicating that the weekly averages do represent a normal mean. This is attributed to the greater blending incidental to large-scale factory operation.

Figure 1 shows graphically that representative samples of raw tomatoes were obtained and that the effect of variations in tomato color on ketchup color can be determined accurately and expressed numerically. Having been obtained for a full production, factory operation, these data lend added significance to those obtained on pilot plant operations where factory equipment may be duplicated, but the timing and effect of quantity production cannot be easily simulated. These data presented in this study lend further significance to already published work, and certainly further indicate the value of the Hunter instrument in studying color of natural products. It is suggested that a similar approach could be used to relate grade color to finished product color on factory-size operations as well as pilot plant scale where it is extremely difficult to accurately simulate production conditions.

Charts similar to Figure 1 were also prepared showing the relation of Hunter *a* and *b* values of tomatoes and ketchup color. Similar relations were found although the differences were not as pronounced as obtained with the calculated Color Index.

Hunter color of pulped graded tomatoes. While the main emphasis of this study was placed on the relation of raw tomato to ketchup color, some data were collected on the color of pulped graded tomatoes. Sufficient data wre not collected to justify the drawing of definite conclusions or making definite recommendations. Tomatoes were graded by the regular inspectors on the grading platform as U. S. No. 1 and U. S. No. 2 fruit on the basis of color only. These graded samples were then pulped and the color determined for each grade. Samples of the pulp from each grade were then blended together in the proportion that each was contained in the raw fruit. Thus the color of each grade was obtained and also the overall color of the whole sample.

Weekly averages of the pulp color for the grades and blends are shown in the chromaticity diagram in Figure 2 *(5, 6)*. A total of approximately 100 samples is represented. In this diagram both Hunter *a* and *b* values are

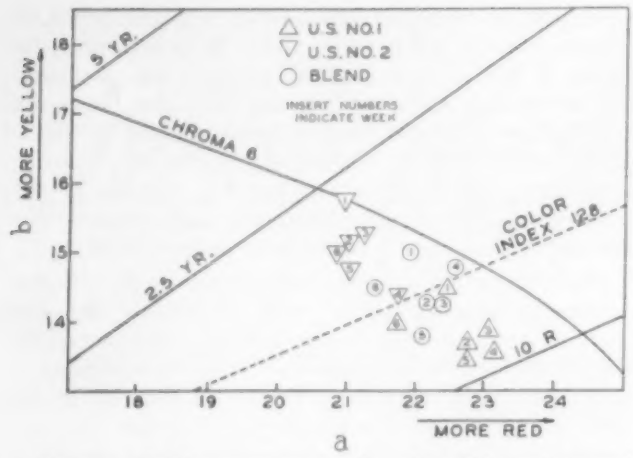

Figure 2. Weekly averages of color of graded tomatoes and blends made therefrom.

shown and as expected, as the red increases, the yellow decreases. Also shown on this chart are the Munsell renotations for hue and chroma at 3/value as compared to the Hunter color units. This relation between the Munsell and Hunter systems has been described in some detail by Younkin *(6)*. The ranges of the grade colors encountered are illustrated and the broken line (Color Index, 1.28) indicates approximately the dividing line between U. S. color grades No. 1 and No. 2 based on the limited data obtained.

As an indication of the possible advantages to be obtained by objectively grading pulped tomatoes, Figure 3 shows graphically the wide divergence existing between visual grading by U. S. Standards and the Hunter Color Index of pulped tomatoes. For example, at a Color Index Number of 1.15, which represents a good color pulp, the PMA visual system graded the raw fruit from approximately 90% No. 1's down to 30% No. 1's for tomatoes which produced the same color pulp. Likewise, for tomatoes that graded approximately 58% No. 1's at various times during the season, the pulp color covered almost the whole range of colors encountered during the whole season. Quite probably this disparity between the 2 methods is due to the fact that PMA grading makes no discernment between high and low No. 1 color, or high and low No. 2 color. According to U. S. Standards, No. 1 grade must have 90% of good red color while No. 2 grade must have between 66⅔% and 90% good red color. The range for No. 2 color is very wide. An arbitrary line at 90% good red color does not indicate the range of color encountered, and does not represent the color of pulped tomatoes. The use of an objective method, such as that presented using the Hunter instrument gives a numerical graduated value for overall tomato color covering the entire range which is not affected by personal factors such as individual differences in ability to see and describe color, fatigue, and lighting conditions at time of examination. The Hunter instrument is completely impersonal.

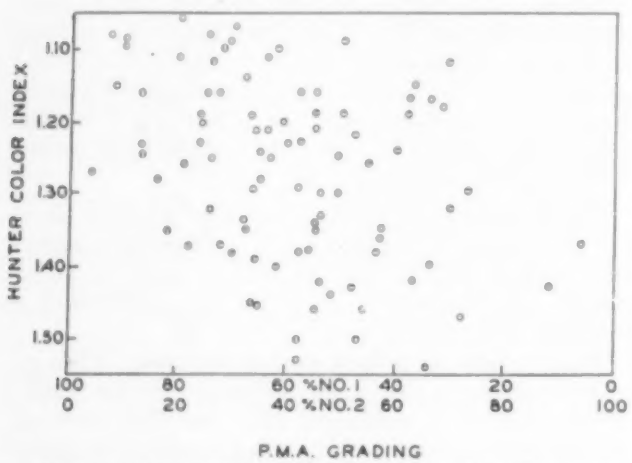

Figure 3. Comparison of PMA grade with Hunter measurement of color of graded tomatoes.

The fact that the Hunter color measurement of pulp gives a more accurate evaluation than the visual grading method principally by surface color seems obvious. However, the practicality of such objective color measurement for grading platform use is not readily apparent using present techniques of sample selection and preparation. A more rapid method of sample preparation would certainly simplify and make more practical the method for the speed with which commercial grading must be accomplished in practice. These data do indicate the practicality of the information obtained, and suggest the desirability of adopting such a method for commercial grading. Experimental work on tomato grade revision by State and Federal agencies is pointing in the direction of such methods of color grading and the evaluation of color grading methods. Such work, if at all possible, should be conducted on commercial operations as well as on laboratory and pilot plant equipment.

SUMMARY

The results presented show that a surprisingly good relation has been obtained comparing raw tomato color with ketchup color on a full production factory-size operation. The method which utilizes the Hunter Color-Difference Meter gives accurate determinations which can be expressed numerically. Production operations can be studied and reliable data obtained which can be used to supplement and add significance to pilot plant studies. It is suggested that a similar approach to the relation of grade color to finished product color would be of great value in the promulgation of more realistic standards.

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The Bacteriology of Gulf Coast Shrimp. IV. Bacteriological, Chemical, and Organoleptic Changes With Ice Storage[a]

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(Manuscript received December 26, 1951)

Headed, washed shrimp in crushed ice retained acceptable commercial quality for at least 16 days. There was an increase in Achromobacters, and in the several chemical substances for which changes in amount were determined.

A major problem in the commercial handling of food is the prevention of deteriorative changes which will make the food unacceptable to a discriminating consumer. While in some instances only reasonably prompt transit is needed, in others the nature of the product and the conditions of production or final fabrication make it obligatory that more elaborate precautions be observed. The changes which take place are due to the enzymes naturally present in the food stuff, and the action of microorganisms ordinarily present or which may be introduced at some stage of processing. The methods of control or prevention of enzymatic and microbiological changes depend upon the nature of the food stuff and the type of commodity. Deteriorative changes in marine products are especially likely to be perceptible at an early stage, and their prevention is of much importance to the industry.

The numerous reports concerned with chemical and microbial changes which occur with time in fish as reviewed by Reay and Shewan *(8)* suggest that the activity of microorganisms is more important than that of the tissue enzymes. It seems reasonable to assume that the tissue enzymes facilitate microbial action by autolytic softening of the tissues, although it is difficult to evaluate independently the part played by each. It is probable that the changes in shrimp are comparable to those in fish, but so far as could be determined no investigations of shrimp similar to those on fish have been recorded. Green *(4)* has reported the quantitative changes in the bacteria of shrimp during ice storage for up to 9 days, and noted the lack of bactericidal worth of germicidal ice. In earlier reports *(6, 10, 11, 12)* methods of examination, quantitative and qualitative observations on the external flora, the intestinal flora and yeast species from shrimp have been discussed. In the present report the results of laboratory experiments on chemical and microbial changes in well-iced shrimp are given. The work was undertaken with the idea that the data developed could perhaps be translated through pilot operations into commercial application, and result in improved methods of handling with consequent lessened loss because of deterioration before processing.

[a] This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.

EXPERIMENTAL

Shrimp of about 30-40 count (30-40 per pound) were taken from the deck immediately after emptying from the trawl, headed, washed thoroughly with sea water, and packed in crushed ice in a Shamrock packer for transport to the laboratory. During storage the shrimp were held in an abundance of crushed ice. They were kept from mixing with the water of the melted ice by a false bottom of hardware cloth.

Bacteriological, chemical, and organoleptic examinations were made at intervals up to 16 days. The bacteriological examination consisted of total counts of the external flora, and quantitative and qualitative determinations of the genera represented. Shell-free tissue samples for chemical examination were comminuted in a Waring blendor, and the following tests were made.

Dimethylamine was determined by the procedure of Dowden *(2)*, trimethylamine by the colorimetric method of Dyer *(3)*, and amino nitrogen by the method of Pope and Stevens *(7)* using the filtrate from a 50-g. sample comminuted for 5 minutes with 50 ml. of water and 100 ml. of 10% trichloracetic acid.

Volatile acids were titrated with 0.01 *N*. sodium hydroxide, using phenolphthalein as an indicator, in 100-ml. fractions collected by steam distillation of 35 g. of tissue in a mixture of 15 ml. of 8 *N*. sulfuric acid, 50 ml. of 10% phosphotungstic acid solution, and 28 g. of magnesium sulfate.

Peroxide values were measured by the method of Wheeler *(9)* and free fatty acids by the A.O.A.C. procedure *(1)* using an ether extract obtained by shaking for 1 hour on a mechanical shaker a mixture of 50 g. of tissue, 50 g. of anhydrous sodium sulfate, and 200 ml. of ethyl ether.

Hydrogen sulfide was tested for by the technique of Nickerson, Goldblith and Proctor *(5)*.

RESULTS

The data which have been obtained can be given best in tabular form. Table 1 shows the changes with time in total count, and Table 2 gives the percentage distribution among the principal genera found. Table 3 records the chemical and organoleptic changes.

It will be noted that there was a reduction in the 30° C. (86° F.) counts at the 4 and 8 day intervals. This is believed to be due to the washing effect of the melting ice. The increase in counts at 12 and 16 days

TABLE 1
Bacterial counts on Gulf shrimp held in crushed ice[b]

	Days held in storage				
	0	4	8	12	16
Ranges at 30° C.	12,000-60,000	4,000-25,000	7,000-35,000	140,000-500,000	6,300,000-25,000,000
Average counts at 30° C.	25,400	8,500	11,000	250,000	10,000,000
Ranges at 8° C.	.....	.....	.....	130,000-600,000	8,000,000-29,000,000
Average counts at 8° C.	.....	.....	.....	242,000	12,000,000

[b] Per gram of tissue.

can be accounted for by the increase in numbers of organisms growing at 8° C. (46.4° F.). In the earlier examinations the organisms growing at the low temperatures were too few to be detected by the dilutions cultured and the time of incubation used. Obligately psychrophilic bacteria have not been encountered, consequently the organisms developing at 30° C. (86° F.) include both those which can grow at 8° C. (46.4° F.) and those which cannot. Not all of these can grow at the lower temperature, hence the difference in counts.

TABLE 2

The changes in bacterial flora of Gulf shrimp during storage in crushed ice [c]

Days held in storage	Achromobacter	Bacillus	Flavobacterium	Micrococcus	Pseudomonas	Miscellaneous
0	27.2	2.0	17.8	33.6	19.2	0.2
4	31.3	0.6	13.1	23.0	26.5	0.5
8	46.0	2.0	18.0	5.7	28.0	0.3
12	67.0	0.0	2.0	0.8	30.1	0.1
16	82.0	0.0	1.5	0.0	16.5	0.0

[c] Expressed as percentage of total number of organisms isolated at each time interval.

TABLE 3

Organoleptic quality of and chemical tests made on Gulf shrimp held in crushed ice

Test	Days of storage				
Organoleptic quality	0	4	8	12	16
	Excellent	Excellent	Good	Good	Fair
Dimethylamine nitrogen [d]	.....	0.55	0.93	2.11	3.19
Trimethylamine nitrogen [d]	.....	0.54	0.70	1.82	2.33
Amino nitrogen [d]	.....	45.13	59.64	70.88	95.18
Volatile acids [e]	.....	5.23	6.36	7.18	9.24
Free fatty acids [f]	.....	0.27	0.67	0.85	1.35
Peroxide No.	.....	0.00	0.00	0.12	0.36
Hydrogen sulfide	.....	0.00	0.00	0.00	Trace

[d] Mg. per 100 g. tissue.
[e] Ml. 0.01 *N*. NaOH per 100 g. tissue.
[f] Percent as oleic acid.

Table 2 gives the shift in percentage of each genus with time of storage, and throws further light on the data of Table 1. In earlier work it was found that the organisms growing at low temperature were largely Achromobacter, and that most species of this genus grew well at temperatures as low as 4° C. (39.2° F.). Table 2 shows a steady increase in percentage of Achromobacter to a marked domination of the flora. The ability of Achromobacter to develop slowly in ice storage, and the well established significance of this genus as a cause of spoilage in fresh foods, are certainly limiting factors in the preservation of shrimp by ice storage.

The first chemical determinations were made after 4 days of storage. Increases in amount with the passage of time of each substance tested for were noted. Further work must be done, though, to relate any one of the tests to quality deterioration. The absence of peroxide in detectable amounts before 12 days, and the appearance of hydrogen sulfide only after 16 days may deserve closer attention. The increase in amino nitrogen with time likewise may have more than casual interest.

Tests were made for indol irregularly, but negative results were recorded except in cases where the shrimp were definitely undergoing decomposition.

The designations of organoleptic quality given in Table 3 are based on examinations of a cooked sample. Even after 16 days of storage the shrimp were edible, although not as well flavored as initially or when examined after shorter storage. The difference in flavor between shrimp held 8 days from those held 12 and 16 days was not great, but was definite enough to suggest that ice storage of less than 12 days is desirable. However, it is emphasized that there was no development of unsatisfactory texture or appearance, or disagreeable odor noted in the shrimp stored 16 days. It is believed that these were still of acceptable commercial quality.

Parallel experiments were made with the shrimp in glass jars packed in crushed ice, and dry ice storage. It has long been an observation of the industry that contact of the shrimp with melting ice is important in delaying deterioration, and the laboratory observations support the practical observations. The shrimp in glass jars, packed in an abundance of crushed ice, had a very short storage life. Dry ice was not satisfactory due to rapid deterioration of texture and appearance.

SUMMARY

Laboratory experiments on the storage of fresh shrimp in crushed ice indicate that satisfactory commercial quality can be retained for at least 16 days, although storage for less than 12 days appears to be desirable. The shrimp should be in contact with the melting ice.

There is an increase in psychrophilic bacteria with time sufficient to account for the total increase in bacteria. Achromobacter becomes the dominant genus.

There was an increase in amount of each of a number of chemical substances, but further work is needed to relate any one of these to organoleptic quality.

Acknowledgment

The authors wish to thank Mr. J. L. Baughman, Chief Marine Biologist of the Texas Game, Fish, and Oyster Commission for his cooperation in this work. He made available freely the facilities of the Rockport Laboratory of the Commission, and by so doing greatly facilitated the investigation.

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Development of Off-Flavor in 20° Brix Orange Concentrate Inoculated With Certain Strains of Lactobacilli and Leuconostoc[a]

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(Manuscript received December 22, 1951)

The development of off-flavor in 20° Brix orange concentrate was studied by inoculating the orange concentrate with different strains of Lactobacilli and Leuconostocs. The time required for the development of off-flavor for the different strains at different initial levels of the inoculum has been determined.

Frozen orange juice concentrate, as it is generally produced, is not heat sterilized and, therefore, is not bacteriologically sterile. Of necessity, it is handled with great rapidity to minimize certain chemical and physical effects which may cause undesirable changes in flavor and consistency. Rapid handling also permits control of the growth of microorganisms so that in efficient plant operations there is ordinarily no appreciable buildup in total counts between the juice extractors and the frozen concentrated product in the sealed cans.

In view of the expanding demand for frozen orange juice concentrate, packers must consider all factors affecting quality in order to assure a continuing market in the future. From the bacteriologist's viewpoint, this means that extreme caution and careful supervision of bacteriological control must be constantly maintained over the manufacturing and canning operations to keep bacterial contamination of the product to a minimum.

The control of and a substantial elimination of a buildup of microorganisms capable of producing off-odors and off-flavors in frozen orange juice concentrate is probably the most important problem to the packer. Failure to prevent a buildup of these organisms in the product may result in serious economic losses. The 2 prevalent types of bacteria known to cause off-flavors in orange concentrate are Lactobacilli and Leuconostocs. Previous studies conducted by our laboratories have shown that certain strains of these organisms will grow actively and produce an off-flavor in single strength orange juice and 20° Brix orange concentrate. One strain of Lactobacilli also grew and produced an off-flavor in 42° Brix concentrate at 30° C. Packers of frozen concentrated orange juice, through their bacteriological control procedures, have found that these types of bacteria may be present in varying concentrations in their product and have realized that unless certain precautionary measures are observed in their manufacturing operations, spoilage may result. Hays *(2)* describes certain strains of Lactobacilli which were responsible for off-flavor production of orange concentrate. Consequently, packers are extremely interested in knowing what population level of Leuconostoc and Lactobacilli organisms can be present in the juice or concentrate and not be considered detrimental to quality. This study was conducted to assist the packers in finding an answer to this problem.

[a] Presented at the meeting of the Florida State Horticultural Society, West Palm Beach, Florida, October 29, 1951.

Based on previous data which showed the majority of the test organisms grew actively in 20° Brix orange concentrate at 20° C. (68° F.), the approximate Brix and temperature of the product in the first effect of many evaporators; it was decided to study the growth and development of off-flavors in this product at that temperature and stage of concentration. It was also considered that this was a critical point in the concentration of orange juice with respect to the growth of bacteria. Beisel *(1)* observed that acid formers are capable of rapid growth under suitable conditions, particularly within the first stage of concentration.

EXPERIMENTAL PROCEDURE

Bacteriological studies in connection with this project were conducted both, in the laboratory and in several citrus concentrate plants involving the use of a large number of test organisms. However, the data reported herein were based on the use of only 12 organisms, 9 strains of Leuconostoc and 3 strains of Lactobacilli. All of these strains had been found to produce off-flavors in 20° Brix orange concentrate and the data reported are typical of the results obtained with the majority of the other organisms. Two of the Leuconostoc strains were *Leuconostoc mesenteroides*, one strain obtained from Dr. Reese H. Vaughn of the University of California, and one strain received from Dr. F. W. Fayville of the Citrus Experiment Station, Lake Alfred, Florida. Seven other strains isolated in our laboratory, although not definitely identified as *Leuconostoc mesenteroides*, but considered to be of the Leuconostoc type based primarily on their gum forming properties, their ability to ferment sucrose and pentoses, and their morphology, were included in the studies reported herein. All of the Lactobacilli strains were obtained from Dr. Carl S. Pederson of the New York State Agricultural Experiment Station, Geneva, N. Y.

The strain number, species, and source of the test organisms are listed below:

Strain No.	Test organism	Source
851	*Leuconostoc mesenteroides*	Dr. R. H. Vaughn, University of California
894	*Leuconostoc mesenteroides*	Dr. F. W. Fayville, Citrus Exp. Station
803	*Leuconostoc sp.*	42° Brix orange concentrate (normal)
856	*Leuconostoc sp.*	42° Brix orange concentrate (normal)
868	*Leuconostoc sp.*	42° Brix orange concentrate (normal)
869	*Leuconostoc sp.*	42° Brix orange concentrate (normal)
870	*Leuconostoc sp.*	42° Brix orange concentrate (normal)
862	*Leuconostoc sp.*	42° Brix orange concentrate (off-flavored)
893	*Leuconostoc sp.*	Brown spot on an orange
879	*Lactobacillus plantarum*	Dr. Carl S. Pederson, Geneva, N. Y.
887	*Lactobacillus sp.*	Dr. Carl S. Pederson, Geneva, N. Y.
890	*Lactobacillus sp.*	Dr. Carl S. Pederson, Geneva, N. Y.

Bacterial suspensions were prepared from actively growing cultures of the test organisms in 20° Brix orange concentrate (pH 3.5, total acidity 1.5% as citric acid) at 20° C. The production of off-flavor was determined by inoculating a series of screw cap tubes (25 mm. x 150 mm.) containing 25 ml. of sterile 20° Brix orange concentrate with 0.1 ml., 0.5 ml., and 1.0 ml., respectively, of a 1 to 10 dilution of each suspension. Then 1 ml. of product from each tube of each level of inoculum was plated immediately, the remainder was reconstituted and checked organoleptically for off-flavors. Similar examinations were made daily over a period of 4 to 7 days. Bacterial counts were

obtained at the time of each examination by plating on orange serum agar, pH 5.4, and counting after 4 days of incubation at 30° C. The reconstituted juice was checked for off-flavor by a tasting panel composed of 3 members of the Research Laboratory staff using the following rating scale: 10, normal flavor; 8, slightly off-flavor; 6, distinctly off-flavor; and 0, obnoxious.

RESULTS AND DISCUSSION

An example of some of the data obtained by the test organisms studied is presented in Table 1, which showed the production of off-flavor in 20° Brix orange concentrate by Leuconostoc strain No. 856. It will be noted that 3 different levels of inoculum were used: 55,200, 310,000, and 600,000 bacteria per ml. The lowest level of inoculum (55,200 bacteria per ml.) required 3 days of incubation at 20° C. (68° F.) to obtain a flavor rating of 6 or less. The plate count was 26,900,000 bacteria per ml. The other 2 levels of inoculum (310,000 and 600,000 bacteria per ml.) required 2 days of incubation to produce an off-flavor rating of 6 or less with plate counts of 9,500,000 and 22,900,000 bacteria per ml.

Table 2 is representative of 6 of the 9 Leuconostoc strains investigated and showed the number of days required to produce an off-flavor in 20° Brix orange concentrate incubated at 20° C. (68° F.). Initial inoculation levels of 100 to 3,700,000 bacteria per ml. were employed.

It is interesting to note that strain No. 803 using an initial inoculum of 200 bacteria per ml. required 7 days to produce a flavor rating of 6 or less with a resulting count of 144,000,000 bacteria per ml. Increasing the level of inoculum of the same organism to 1,400 bacteria per ml. reduced the time required to produce a distinct off-flavor to 4 days with a resulting count of 53,000,000 bacteria per ml. In contrast, strain No. 851 with an initial inoculum of 100 bacteria per ml. required only 4 days to produce a flavor rating of 6 or less with a count of 8,000 bacteria per ml. Increasing the inoculum level of this test organism to 700 bacteria per ml. reduced the time to produce a distinct off-flavor to 3 days with a resulting count of 130,000 bacteria per ml. Two strains, No. 869 and No. 870 (the latter strain not shown in Table 2) did not grow when inoculated into 20° Brix orange concentrate. In fact, a reduction of 30 to 35% in bacteria counts occurred during incubation. However, both strains produced a definite off-flavor within 2 to 3 days. The development of off-flavor in this instance may have resulted from end products of metabolism or to enzymatic action by the bacteria.

The data indicated that Lactobacilli grew better in 20° Brix orange concentrate than Leuconostocs. For example, strain No. 887, using an inoculum level of 4,500 bacteria per ml. increased to 1,060,000,000 bacteria per ml. after 7 days of incubation at 20° C. (68° F.).

Table 3 showed the number of days required for 3 strains of Lactobacilli to produce a definite off-flavor in 20° Brix orange concentrate at 20° C. (68° F.). The levels of inoculum ranged from 4,500 to 3,500,000 bacteria per ml. A flavor rating of 6 or less was obtained in 1 to 4 days of incubation with resulting counts of 70,200,000, 8,000,000, and 11,700,000 bacteria per ml., respectively.

The development of off-flavor in 20° Brix orange concentrate inoculated with various strains of Leuconostoc and Lactobacilli and incubated at 20° C. (68° F.) was found to be dependent upon the following factors: 1, the strain of Leuconostoc or Lactobacilli used as the test organism; 2, the initial level of inoculum; and 3, the period of incubation.

The data indicated that no definite population level could be taken as a dividing line between safe and dangerous operating conditions with respect to the development of off-flavors in 20° Brix orange concentrate by these types of bacteria.

Apparently, based on "normal" contamination, considerable time is required for an off-flavor to develop in orange concentrate in the first effect of the evaporator [approximately 20° Brix (65-68° F.)] as the direct result of the growth of Leuconostocs and Lactobacilli. The data obtained indicated that it may require from

TABLE 1

Production of off-flavor in 20° Brix orange concentrate incubated at 20° C. Leuconostoc strain No. 856

Days of incubation	Test 1		Test 2		Test 3	
	Bacteria per ml.	Flavor [b] rating	Bacteria per ml.	Flavor rating	Bacteria per ml.	Flavor rating
0	55,200	10	310,000	10	600,000	10
1	....	10	920,000	10	1,400,000	9
2	9,360,000	8	9,500,000	6	22,900,000	3
3	26,900,000	0	38,600,000	0	67,500,000	0
4	6,800,000	0	9,000,000	0	34,000,000	0

[b] Flavor rating: 10, normal; 8, slightly off; 6, distinctly off; and 0, obnoxious.

TABLE 2

Number of days required for Leuconostoc to produce an off-flavor in 20° Brix orange concentrate incubated at 20° C.

Strain No.	Test No.	Bacteria per ml., after inoculation, flavor rating: 10	Bacteria per ml., flavor rating: 6 or less	Time in days to reach flavor rating of 6 or less
803	1	200	144,000,000	7
	2	1,400	53,000,000	4
851	1	100	8,000	4
	2	700	130,000	3
856	1	55,000	26,900,000	3
	2	310,000	9,500,000	2
	3	600,000	22,900,000	2
868	1	384,000	1,200,000	2
	2	3,700,000	4,400,000	2
869	1	4,300	1,000	5 [c]
	2	33,000	10,000	3
	3	110,000	38,000	2
893	1	17,000	3,900,000	7 [c]
	2	105,000	4,000,000	3

[c] Flavor rating of 6 or less was not obtained; a rating of 7 or 8 resulted at the time designated.

TABLE 3

Number of days required for Lactobacilli to produce an off-flavor in 20° Brix orange concentrate incubated at 20° C.

Strain No.	Test No.	Bacteria per ml., after inoculation, flavor rating: 10	Bacteria per ml., flavor rating: 6 or less	Time in days to reach flavor rating of 6 or less
879	1	201,500	11,700,000	4
	2	680,000	1,330,000	2
	3	1,570,000	5,500,000	2
887	1	4,500	8,000,000	4
	2	26,000	8,200,000	2
890	1	376,000	260,000,000	2
	2	3,500,000	70,200,000	1

one to several days to produce off-flavors depending largely upon the strains of bacteria and the degree of contamination present in the product. In some types of evaporators in which recirculation is used, it is possible for some juice to remain in the first effect for unusually long periods of time.

Experience has indicated the condenser to be the most likely point for a buildup of contamination with these types of bacteria, particularly if this equipment is not kept clean. Normally, microorganisms in the condenser would not gain access to the product in the evaporator; however, a drop in the vacuum or fluctuation of pressure in the system could result in splashing back of condensate from the condenser into the evaporator.

The data which we obtained showed that strains of certain species of organisms may, if present in sufficient numbers, produce an off-flavor in 20° Brix concentrate in 24 to 72 hours. Under commercial operating conditions, in order to avoid difficulties from this source, it is advisable that all practical precautions to insure a low initial contamination of the raw juice entering the evaporator be maintained, and frequent and thorough cleaning of the evaporating equipment be consistently practiced.

The importance of an effective sanitation program has been stressed by a number of investigators. Beisel *(1)* states the frequency of cleaning the evaporator is determined by their basic design with most units requiring cleaning every 72 hours. A well-designed evaporator according to Schwartz *(3)* will allow operation on citrus juices for periods as long as 130 hours with no significant increase in bacterial count. However, the use of soft fruit or a poor sanitation program may require cleaning every 72 hours or more often. Bacteriological checks should be taken at scheduled intervals several times a day to detect the presence of a buildup of organisms normally associated with off-flavor production. A significant buildup should be taken as an indication for immediate shut-down and thorough cleaning. Occasionally, in the past, evaporators have been operated continuously for 10 days to 2 weeks without a thorough clean-up. It is obvious from the data presented that this is not a safe practice.

SUMMARY

A study was made of the development of off-flavor in 20° Brix orange concentrate incubated at 20° C. (68° F.) inoculated with 9 strains of Leuconostocs and 3 strains of Lactobacilli. An average of 3 days' incubation at 20° C. (68° F.) were required to produce a definite off-flavor. The production of off-flavors was dependent upon: 1, the strain of Leuconostocs and Lactobacilli; 2, the initial level of inoculum; and 3, the period of incubation at 20° C.

The higher the initial population level or contamination of the product, the shorter was the incubation period required to produce an off-flavor.

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Effect of Subtilin and Streptomycin on the Formation of Nitric Oxide Hemochromogen in Cooked Ground Fresh Beef

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In cooked fresh meat a red discoloration may occur which has been attributed to the presence of nitrate which has been reduced to nitrite. The nitrite can combine with myoglobin to form nitric oxide myoglobin, a pigment which has been shown to be identical with the pigment which gives unheated cured meats their characteristic color. This investigation was inaugurated to explore some of the possibilities for the study of this undesirable red discoloration. The specific phase investigated was whether the antibiotics subtilin and streptomycin had an effect on the prevention of the formation of nitric oxide hemochromogen in cooked ground fresh beef.

The necessity for the thorough cooking of meats, especially pork, has been impressed upon the homemaker and those responsible for food preparation. A general rule for the indication of the doneness of meat is the absence of pink or red color in the meat and the presence of a brownish or grayish color which develops with the application of heat upon the unstable red heme substances in the muscle tissue. Consequently, perplexity has resulted whenever meat in various dishes has retained a pink or red color which persists even after prolonged cooking. In roasts the red discoloration usually occurs as a band near the surface of the meat, but in loaves made of fresh ground meat, it may color the entire loaf. The resulting product which has the appearance of raw muscle is unappetizing and presents a problem in food acceptance.

Previous investigations have shown that the red discoloration in cooked fresh meat is usually due to the action of nitrates or nitrites on myoglobin which results in the formation of nitric oxide hemochromogen. In his analysis of the nitrates and nitrites in foods Richardson *(16)* observed that small pieces of cubed beef stewed with certain fresh vegetables became discolored by the formation of "nitrosohemochromogen" in the same manner as when boiled with nitrate-cured bacon or with a dilute solution of nitrite. Jensen reported this description *(8)* of the red discoloration and other causes *(8, 9)* for its occurrence. While there is no objection to the

controlled production of nitric oxide pigments in cured meats, there is a question in regard to its presence in cooked fresh meat mainly because the reduction of the nitrate may be brought about by bacterial action. Precise information about the ingredients in the formula, storage conditions, and the bacterial load of the meat loaf which may influence the development of discoloration is not in published form and nearly all of the available references on the mechanism of the formation of nitric oxide myoglobin are those in relation to its production in cured meats.

Haldane *(6)* investigated the development of color in meat with nitrate, Hoagland *(7)* pointed out the likelihood of the reduction of nitrate as an intermediate step in the formation of the nitric oxide derivative, and Jensen *(10)* made detailed studies of heme pigments including that of nitric oxide hemoglobin. In the preparation of nitric oxide hemoglobin Urbain and Jensen *(18)* concluded that the properties of myoglobin and hemoglobin were sufficiently alike to substitute blood as a source of heme in their experiments. Duisberg and Miller *(4)* in their study of color development in cured pork also made a distinction between myoglobin and hemoglobin. The red pigment of muscle tissue is muscle hemoglobin or myoglobin *(9)*. References on the reactions of nitric oxide and myoglobin and of the subsequent product, nitric oxide myoglobin (which upon the application of heat becomes nitric oxide hemochromogen) are made by Jensen *(9)* and Niven *(15)*.

Other workers *(4, 5, 13, 14)* have reported the role of the ingredients, nitrate, nitrite, salt, and sugar in the meat curing process and the role of pH, bacteria, and oxygen tension in the formation of nitric oxide myoglobin. Richardson *(16)* and Tanner *(17)* commented that the role of nitrate in meat cures is not solely one of color fixing, but also that of a preservative, since nitrate-nitrite-salt-sugar cures favor the growth of most aerobic bacteria and inhibit putrefaction by anaerobic organisms. Jones *(12)* has shown that salt exercises selective action as it functions as a preservative in meat cures. It, also, produces conditions which are unfavorable for the growth of putrefactive organisms but does not prevent the activity of nitrate-reducing organisms.

Early work on the tissue of normal animals pointed to the fact that muscle tissues are sterile. However, the matter is controversial and Jensen and Hess *(11)* and Tanner *(17)* report that there is definite evidence that meat tissues may not always be uncontaminated by bacteria. The latter referred to studies in which many bacteria were found to be able to reduce nitrate readily to nitrite. It was also found that, in the preservation of meats, the nitrate must be reduced to nitrite before the color may be fixed. Since bacteria are active in reducing nitrates, they probably play the most important role in this respect in meat cures.

On the basis that nitrate is a direct factor in the red discoloration of cooked fresh meat and that the reduction of the nitrate could be of bacterial origin, the two antibiotics, subtilin and streptomycin were employed in the experimental preparation of meat loaves.

In previous work on the use of antibiotics as possible food preservatives, the emphasis was upon their use in connection with canned vegetables and canned comminuted meats *(1, 3)*. In this study the problem involved was whether or not the simple direct addition of subtilin or streptomycin to a meat sample (containing sufficient nitrate to produce discoloration) would prevent the development of the characteristic pigment, nitric oxide hemochromogen.

TABLE 1

Bacterial count at 1 to 1000 dilution on raw and cooked samples of ground beef baked immediately, stored in the refrigerator, and stored at room temperature

Sample No.	Baked immediately		Stored			
			In refrigerator for 24 hours		At room temperature for 24 hours	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Control	19[c]	0	30	0	187	0
	0	0	0	0	TN[a]	0
	0	0	0	0	TN	0
Nitrate	20	0	0	0	389	1
	1	0	0	0	TN	0
	0	0	2	0	TN	0
	0	0	0	0	500 (est.)	0
Subtilin	2	0	0	0	20	0
	0	0	0	0	TN	0
	0	0	0	0	423	0
	..	..	..	..	107	0
Subtilin-nitrate	10	0	2	0	TN	0
	3	0	0	0	TN	0
	1	0	0	0	TN	0
	0	0	0	0	414	0
Streptomycin	..	..	..	..	0	0
	..	..	..	..	1	0
	..	..	..	..	..[b]	0
	..	..	..	..	36	0
	..	..	..	..	44	0

[a] TN, too numerous to count.
[b] (c), contaminated by mold.
[c] Count per plate at 1 to 1000 dilution.

EXPERIMENTAL METHODS

The meat for each set of samples was obtained in a single lean piece from which the outer portion was trimmed and discarded. It was ground and handled with sterile equipment in order to keep the bacterial load at a minimum. Chemically pure nitrate was used in a solution of known concentration and the celery was a fresh Pascal variety. The subtilin was contributed by the Western Regional Research Laboratory from Lot 326 and the streptomycin was in the hydrochloride and dihydrosulfate forms.

Each 50 g. sample was mixed, stored, and baked individually in pyrex containers. The baking was done in a Dispatch rotary-floor oven with constant temperature control set at 325° F. (163° C.) for a period of 25 minutes.

Preliminary steps were taken to determine the levels of nitrate, subtilin, and streptomycin to be used in the experimental work. With these levels established, meat samples were prepared to which the various additions were made. The samples included controls of plain ground fresh beef and samples of beef plus one of the following: nitrate, subtilin, subtilin and nitrate, streptomycin and nitrate, celery, or streptomycin and celery.

Samples were baked immediately, stored 24 hours at refrigerator temperatures and baked, or stored 24 hours at room temperature and baked. A storage period was found to be necessary to allow for the reduction of the nitrate to nitrite and all subsequent samples were held 24 hours at room temperature. After the storage period a 10 g. portion was removed from some of the meat samples before and after baking for the purpose of making pour plates, which had been suggested as an indication of the bacterial load. The pour plates were made according to the technique outlined in the American Journal of Public Health *(2)*, incubated 48 hours at 37° C., and counted at the 1 to 1000 dilution.

Subjective measurement of the color of the meat samples after baking was made and objective measurement of some of the samples was made by the use of the Hunter Color Difference Meter.

RESULTS AND DISCUSSION

In this investigation the development or the absence of the permanent red discoloration was the basis for judging the effectiveness of the antibiotics. In regulating the other factors which would influence the development of the nitric oxide myoglobin several exploratory steps were taken as described below.

Samples were treated with different amounts of nitrate to determine the level at which the discoloration was produced consistently. The trial quantities were 42, 85, 160, and 240 mg. of nitrate per 50 g. of meat. The smallest amount produced uneven discoloration and the 2 largest amounts did not appear to produce a greater degree of discoloration than that which developed with 85 mg. Consequently, it was decided arbitrarily to use that level throughout the experiment. It was felt that there was reasonable assurance that this level was critical.

The storage time and conditions affect the development of discoloration. None of the samples showed evidence of the formation of nitric oxide hemochromogen when they were baked immediately. Samples stored at refrigerator temperature for 24 hours did not develop discoloration. Storage of samples for 24 hours at room temperature caused the development of distinct red discoloration in the interior and on the surface of the sample. Again an arbitrary decision was made to use storage conditions at room temperature for a period of 24 hours for subsequent samples.

This passage of time was to permit reduction of the nitrate, a change probably due to nitrate-reducing bacteria. A comparison of the bacterial counts on the raw samples seemed to indicate considerable growth in the number of organisms during the period of storage. The meat had been handled carefully and representative counts on the raw samples of the controls, nitrate, subtilin, and subtilin plus nitrate-treated samples were relatively low. Counts on samples stored at refrigerator temperatures did not increase except in one of the control samples and this was negligible. In 12 of the 14 samples stored at refrigerator temperatures, there was no growth on the pour plates at the 1 to 1000 dilution and when cooked these samples showed no discoloration. The bacterial counts on the various samples are shown in Table 1.

The number of organisms on the plates from samples stored at room temperature was generally too high to make an accurate count. The count ranged from 20 to an estimated 500 colonies and above. The samples with high bacterial count were greatly discolored except those without nitrate added. In the streptomycin samples the count was less than 50 colonies per plate in the 5 samples plated and none of these exhibited discoloration when cooked. The plating of cooked samples was suggested as a check, but since nearly all samples were sterile after baking, the counting of colonies was of no practical value.

With the level of nitrate and the time and storage conditions established, work proceeded to the use of subtilin and streptomycin. When subtilin was added at 3 times the highest level which had been used in reports in the literature, 66 p.p.m. discoloration occurred. The amount of subtilin was increased to 135 and 400 p.p.m. and discoloration persisted in all samples in spite of the massive concentration of the antibiotic. The effect of subtilin on the formation of nitric oxide hemochromogen in cooked ground fresh beef is shown in Table 2. The control samples without nitrate were the only ones which did not develop discoloration. Of the nitrate samples 5 were light red and 2 very red. The subtilin-nitrate samples varied from pink to very red. There was no indication from the samples that subtilin had any inhibitory effect on the formation of nitric oxide myoglobin.

TABLE 2

Effect of subtilin on the formation of nitric oxide hemochromogen in cooked ground fresh beef stored 24 hours at room temperature

Sample No.	Control	Nitrate	Subtilin	Subtilin-nitrate
1	ND[4]	2	2	2
2	ND	2	1	2
3	....	2	....	2
4	ND	2	1	4
5	....	2	....	4
6	ST	4	4	4
7	....	4	....	4

[4] ND, no discoloration; ST, slight tinge; 1, pink; 2, light red; 3 medium red; 4, very red; and ...., no observation made.

Additions of streptomycin from 25 units to 500 units per g. of meat were made to nitrate samples. The sample with 500 units of streptomycin per g. showed no discoloration, with 300 units the surface of the sample was gray although the interior was red and with 200, 100, 50, and 25 units the samples showed progressively greater discoloration. On the basis of these results streptomycin was added to the samples listed in Table 3 at the level of 500 units per g. of meat.

The control samples of the study shown in Table 3 were generally without discoloration. Of the nitrate-treated samples 12 were very red and 2 showed only slight development of the red color. Streptomycin prevented the formation of red discoloration in 8 out of 15 nitrate-treated samples. In 5 of the others there was a slight tinge of red, one of the samples was definitely pink and another was medium red.

The streptomycin-nitrate samples were treated identically. Samples 1, 2, and 3 were prepared the same day, samples 4, 5, and 6 on another, samples 7, 8, and 9 on another and samples 10, 11, 12, 13, 14, and 15 on another day. The lack of uniformity may be attributed to a number of factors which are discussed later.

The addition of other food substances to enhance flavor or to serve as extenders is a common practice in the preparation of meat loaf. In the literature *(8, 9, 16)* the nitrate content of 20 g. of fresh celery is approximately the amount of nitrate which had been added to the experimental samples, namely, 1700 p.p.m. Sets of samples with additions of 20 g. and 2 g. of celery were prepared with and without the addition of streptomycin. The results of discoloration are shown in Table 3.

The 20 g. celery samples with streptomycin (with the exception of 1) were discolored. Samples with 2 g. of celery were pink and light red. In some of the pink samples, the discoloration was uneven or spotty. Streptomycin prevented the formation of discoloration in 6 out of 9 of the 2 g. celery samples.

Again, the prevention of discoloration was not com-

TABLE 3

Effect of streptomycin on the formation of nitric oxide hemochromogen in ground fresh beef stored 24 hours at room temperature

Sample No., 50 g.	Control	Nitrate	Streptomycin-nitrate	Celery, 20 g.	Streptomycin-celery, 20 g.	Celery, 2 g.	Streptomycin-celery, 2 g.
1	ND[a]	4	ND	2	2	2	1
2	ND	4	ND	2	2	2	1
3	ST	4	3	3	ND	2	ST
4	ND	4	1	....	....	....	....
5	ND	4	ST	....	....	....	....
6	ND	4	ST	....	....	....	....
7	ND	4	ND	....	....	....	....
8	ND	4	ND	....	....	....	....
9	ND	4	ND	....	....	....	....
10	ND	....	ST	3	1	1	ND
11	ND	1	ST	3	1	1	ND
12	....	1	ST	3	1	1	ND
13	....	4	ND	3	2	1[f]	ND
14	....	4	ND	3	2	1[f]	ND
15	....	4	ND	3	2	1[f]	ND

[a] See footnote [d], Table 2.
[f] The color was uneven pink.

plete, although the streptomycin-treated celery samples did not develop discoloration to the extent of the celery samples. Less discoloration occurred in both sets of celery samples to which streptomycin had been added. Streptomycin treatment seemed to be more effective in preventing discoloration in the 2 g. celery samples than in the 20 g. celery ones. Samples 1, 2, and 3 were prepared at one time and samples 10, 11, 12, 13, 14, and 15 were prepared at another time.

It would appear from the results listed on Tables 2 and 3 that subtilin was ineffective in preventing the formation of nitric oxide myoglobin and thus nitric oxide hemochromogen and that streptomycin was effective in some samples and ineffective in others.

The use of antibiotics with food is in the experimental stage. There is the possibility that further study and replications under different conditions would establish their value more definitely. The first work with subtilin at 5, 10, and 20 p.p.m. by Andersen and Michener *(1)* was promising. Later work by others *(3)* with the level of subtilin at 1 p.p.m. was quite the opposite. Whether or not the lack of success in early work should eliminate the possibilities of the use of subtilin with foods is a question. An increase in the amount of subtilin to a high concentration was not a solution in this study. Subtilin may have been unstable under the conditions of this experiment, since it is a polypeptide and may have been inactivated when added to the meat. The potency of subtilin may have been low in the form in which it was available, or it may not have been active against some or all of the nitrate-reducing organisms.

The success of streptomycin in preventing the formation of nitric oxide hemochromogen in a number of samples is a sign which may indicate that it has possibilities for use in food. It should be noted that the nitrate was added in a comparatively large amount and that the bacterial load was practically at a minimum. Conditions other than those arbitrarily decided upon in this experiment may have produced entirely different results. Further study on storage conditions, innoculation of samples with known nitrate-reducing bacteria, incubation of samples at a constant temperature, or an increase in the amount of streptomycin in samples would have been a feasible approach if more replications of the samples in this study had been possible. Since in the preliminary work 200 and 300 units of streptomycin per g. of meat did not prevent the discoloration and 500 units did prevent discoloration, it is altogether likely that a large amount of streptomycin would be a more appropriate level.

Samples 13, 14, and 15 on Table 3 were read with the Hunter Color Difference Meter. The readings were plotted according to Younkin *(19)* and were in keeping with the results observed subjectively.

SUMMARY

The formation of nitric oxide hemochromogen or its absence was noted by observation and some of the samples were measured objectively with the Hunter Color Difference Meter.

Subtilin at 400 p.p.m. did not prevent discoloration. Streptomycin at a level of 500 "S" units per g. of meat prevented the formation of this pigment in a number of nitrate samples, which would seem to indicate that the reduction of the nitrate was a bacterial origin.

Celery which has a relatively high nitrate content produced discoloration under the conditions stated and is therefore not a recommended ingredient in meat preparations which are not to be cooked promptly.

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The Clarification of Apple Juice by Fungal Pectic Enzyme Preparations[a]

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Several commercial enzymes were analyzed and compared in their effectiveness for the clarification of apple juice. The tests were made on both laboratory and commercial scales. An attempt is reported to correlate the activity of pectic enzymes in such preparations and their effectiveness in clarifying apple juice. Some evidence is presented for the occurrence of two pectic enzymes in apple juice.

In 1930, Mehlitz *(33, 34, 35, 36, 37, 38, 39)* in Germany and Willaman and Kertesz *(19, 20, 49, 50, 51)* in the United States introduced the use of mold pectinases in the clarification of fruit juices. Mehlitz *(35)* recommended the use of mixtures of proteolytic, diastatic, and pectolytic enzymes such as those obtained from *Aspergillus oryzae, Aspergillus wentii,* and malt. Kertesz *(19, 20)* and Willaman and Kertesz *(50, 51)* stressed the use of "pectinases" of *A. niger, A. oryzae, P. glaucum,* and *Rhyzopus sp.*

The enzymic clarification of fruit juices has been studied in the United States by Kertesz *(19, 20)*, Carpenter and Walsh *(6)*, and Marshall *(30, 31, 32)*, and abroad by Atkinson and Strachan *(3)*, Charley *(7, 8)*, Gachot *(12, 13)*, Mehlitz *(33, 34, 35, 36, 37, 38, 39)*, and Weber and Deuel *(47)*. The technological aspects are discussed by Atkinson and Strachan *(3)*, Caldwell *(5)*, Carpenter and Walsh *(6)*, Charley *(7, 8)*, Gachot *(13)*, Hottenroth *(15)*, Kertesz *(19, 20)*, Marshall *(32)*, Tressler, Josyln, and Marsh *(46)*, Tauber *(44, 45)*, Willaman *(49)*, and in other articles *(1)*.

The mechanism of clarification of fruit juices is still not understood. Kertesz *(19)* reported that the cloudiness of juices, particularly apple juice, was partly due to their pectin content, since the colloidally suspended pectin would hold other substances in suspension. He suggested that the principle involved is the conversion of colloidal pectins into particles sufficiently fine to form true solutions. The pectin passing into solution loses its protective action on the other substances and they precipitate out together with insoluble material formed from about 10% of the pectin. He cited the following changes in pectin (determined as alcohol precipitate) during enzymic clarification of cider: the fresh cider contained 0.1840 g. pectin per 100 cc.; after treatment it contained only 0.0560 g. pectin per 100 cc. Carpenter and Walsh *(6)* reported that 1% "Pectinol" removed 44-98% of the pectin (measured as calcium pectate), under similar conditions Clarase removed 34-44% pectin. Others reported similar data with "Pectinol" or "Filtragol" *(34, 35, 36, 37)*.

Enzymic clarification usually is rarely allowed to proceed until the pectic substances are completely hydrolyzed *(21)*. Actually clarification occurs rather early in the hydrolysis. Mehlitz, in his investigation of enzymic clarification, distinguished three phases: 1, destabilization phase, with no visible effect but rapid decrease in viscosity; 2, sedimentation phase, with flocculation; and 3, hydrolysis phase, the complete hydrolysis of pectin.

Our knowledge of the chemistry of pectic enzymes *(21, 22, 23, 26, 40)* and of pectic substances *(21)* has been greatly advanced in recent years, and it was desirable to investigate enzymic clarification from the newer points of view.

Jansen and MacDonnell *(17)* and Jansen, MacDonnell, and Jang *(18)* showed that the polygalacturonase (PG) activity of one commercial fungal preparation (Pectinol) on pectin was dependent on its methoxyl content. They found that the rate and completeness of hydrolysis of pectin by a mixture of polygalacturonase (PG) and pectinesterase was dependent on the rate of hydrolysis of methyl ester bonds by the esterase (PE). If the clarification of apple juice depended upon depolymerization of its pectinic acids, then the effectiveness of pectic enzyme preparations would vary with their relative polygalacturonase (PG) and pectinesterase (PE) activities. For this reason we selected pectic enzyme preparations varying in their PG and PE activity.

EXPERIMENTAL METHODS

Source and composition of enzyme preparations. Commercial enzymes are generally mixed or standardized with various fillers to give products of standard enzyme activity. Dextrose, diatomaceous earth, and gelatin are commonly used. Since these fillers, particularly gelatin, may influence the clarification by affecting both the volume and type of sediment and rate of sedimentation and nature of clarification, it was considered desirable to obtain data on the composition of these enzyme preparations. At the time these tests were undertaken early in 1948, information on this point was not available except for the data obtained on 2 "Pectinol" preparations by Fish and Dustman *(11)*.

The analysis for moisture content, ash, and alkalinity of ash, were carried out by A. O. A. C. methods *(2)*; the total organic nitrogen was determined by a micro-Kjeldahl procedure using the apparatus of Kirk *(24)*; reducing sugars and sucrose were estimated by the ceric sulfate method of Hassid *(15)*. In addition, qualitative tests were made to determine the presence of gelatin, starch, sugar, and diatomaceous earth. The data obtained are shown in Table 1.

[a] Based on a report presented at the annual meeting of the Northern California Section of Institute of Food Technologists, December 8, 1949.

Measurement of pectin enterase (PE) activity. The PE activity was determined by the method of MacDonnell, Jansen, and Lineweaver *(27)*. The reagent solution used consisted of 50 ml. of freshly prepared 1% citrus pectin solution in 0.3 *M*. NaCl, 4 ml. of a freshly prepared enzyme suspension, and sufficient 0.02 *N*. NaOH to adjust the pH to 4.0; this was brought to 100 ml. The final substrate concentration was 0.5% pectin; and the enzyme concentrations varied from 0.5 to 2.0 g. per 100 ml., depending on its activity. Some difficulties were experienced with "Pectinases" as compared to the "Pectinols." It was found that PE activity determinations on "Pectinases" were quite difficult under the conditions of the experiment.

Measurement of pectin polygalacturonase (PG) activity. The PG activity was determined by a modification of the method of Lineweaver, Jang, and Jansen *(25)*. A 5% enzyme solution was prepared by freeing 5 g. of commercial enzyme preparation from interfering reducing sugars by salting out with 25 ml. K_2HPO_4 (saturated solution at 0° C., pH 7), first mixing the enzyme powder to a smooth paste with 5 ml. K_2HPO_4, and then adding the remaining 20 ml. The mixture was placed at 0° C. for 4 to 24 hours and then filtered with suction, using No. 42 Whatman paper. Then the precipitate was washed quantitatively from the funnel and filter paper with 30 ml. of 1 *M*. lactic acid buffer of pH 4 into a 100 ml. volumetric flask and made up to mark with distilled water. Some care had to be taken to make certain that if gelatin was used as a carrier it be brought into suspension before activity determinations were made; otherwise the values found would be low due to adsorption on the carrier. One hour at room temperature (approximately 25° C.) generally was sufficient for this purpose. Where diatomaceous earth was used as carrier, it was found necessary to mix the preparation immediately before removal of the required aliquot.

The pectic acid substrate used was prepared by dissolving 20 g. sodium ammonium pectate (California Fruit Growers Exchange No. 24) in 2 liters of hot (approximately 80° C.) water, using a Waring blendor, and then precipitating the pectic acid by addition of 15 ml. concentrated HCl, added dropwise while stirring continuously. The mixture was poured into a cheesecloth sack (breaking up any large clumps), dialyzed against running tap water for about 1 hour, and then rinsed with distilled water 3 times. The precipitate was removed from the sack and suspended in 500 ml. hot water (80° C.), care being taken to avoid the formation of a stable foam. This suspension was adjusted to pH 4 by addition of 50 ml. 1 *M*. lactic buffer and *N*. NaOH if necessary, and its volume adjusted to 2 liters.

In making the PG activity determination the pectic acid solution preserved by freezing was defrosted and diluted with 0.3 *M*. lactic buffer, pH 4, so that addition of the desired concentration of enzyme at time-zero resulted in a 0.5% concentration of substrate (assuming 85% of the total dry solids were pectic acid). All further steps were carried out precisely as outlined by Jansen and MacDonnell *(17)* in their modification of the hypoiodite method.

Clarification of apple juice. The enzyme preparations, varying in PG and PE activity, described in Table 1, were applied to clarification of freshly pressed apple juice to determine whether high PE was necessary for clarification, as was reported for hydrolysis of pectinic acids of high methoxyl content *(17, 18)*. Then 2 lots of freshly pressed apple juice were treated with these enzyme preparations. The characteristics of these two samples were as follows:

Series	Variety	Date pressed	Condition	Brix[a]	pH	Titratable acidity
						equivalents per liter
I	Gravenstein	Aug. 23, 1949	ripe	15.2	3.5	88
II	Mixture of Rome, Pippin, Golden Delicious	Nov. 14, 1949	very ripe	14.9	3.7	47

During the course of clarification, changes in viscosity, in turbidity, and in color as measured in a Klett-Summerson colorimeter, rate of clarification, volume of sediment, the pectic acid content, free galacturonic acid content, methanol, and tannin content were determined. The methods used for Series I were as follows: pectic acid was determined by the McCready, Swenson, and Maclay method *(29)*; methanol by the A. O. A. C. Denige's procedure *(2)*; and tannin by a modification of the A. O. A. C. method for wine *(2)*. In Series II the free galacturonic acid was determined by a modification of the naphthoresorcinol procedure of Deichman and Dierker *(9)*; methanol by the Bertrand and Silberstein procedure *(4)*; and tannin by the Rosenblatt and Peluso procedure *(43)*.

In conducting the clarification tests, freshly pressed apple juice (standard commercial procedure, pressed at 3000 p.s.i.g.) was quickly filtered with suction through milk filter pads. This removed coarse particles of apple pulp, skin, and core that

TABLE 1

Chemical analysis and activity of the commercial pectic enzymes used

Enzyme No.	Preparation[b] designation	Moisture	Ash			Nitrogen	Glucose	Sucrose	Other filler	[PEu]mg.[c]	[PGu]mg.[d]	PE/PG[e]
			Total	Insoluble	Soluble							
		%	%	%	%	%	%	%				
1	Pectinol A	8.53	1.48	0.89	0.65	1.25	84.8	.....	Gelatin	2.8×10^{-6}	3.6×10^{-4}	0.0078
2	Pectinol M	7.56	1.29	0.52	0.76	0.59	90.0	.....	Gelatin	2.2×10^{-6}	7.8×10^{-4}	0.0027
3	Pectinol 10M	8.72	10.93	8.65	1.05	2.40	60.8	.....	Gelatin	2.3×10^{-5}	6.3×10^{-4}	0.0365
4	Pectinol W	8.90	2.14	1.33	0.80	4.15	63.0	3.0	Gelatin	4.4×10^{-6}	3.6×10^{-4}	0.0122
5	Pectinol W129	8.55	2.72	1.83	0.90	3.68	63.2	3.3	Gelatin	4.7×10^{-6}	4.2×10^{-3}	0.0012
6	Pectinase (R&H)	8.54	27.24	25.13	1.64	4.15	2.5	.....	.....	3.9×10^{-4}	3.9×10^{-4}	1.00
7	Pectinase (W)	4.12	72.09	54.03	18.05	4.35	0.3	.....	Diatomaceous earth	5.6×10^{-5}	1.1×10^{-2}	0.0047
8	Pectinase (T)	8.84	29.75	17.69	11.70	2.95	0.8	0.2	.....	5.5×10^{-6}	2.1×10^{-4}	0.0252
9	Pectinase (W-1)	.....	.....	.....	.....	.....	.....	.....	Diatomaceous earth	1.5×10^{-5}	1.3×10^{-5}	1.15
10	Pectinase (W-2)	.....	.....	.....	.....	.....	.....	.....	Diatomaceous earth	3.9×10^{-6}	1.4×10^{-5}	0.279
11	Pectinase (W-3)	.....	.....	.....	.....	.....	.....	.....	Diatomaceous earth	5.6×10^{-5}	2.0×10^{-4}	0.280
12	Hydrolase 100X	.....	.....	.....	.....	.....	.....	.....	.....	0	0	.....
13	Hydrolase PSP	.....	.....	.....	.....	.....	.....	.....	.....	7.4×10^{-7}	0	.....
14	Pectinol B	.....	.....	.....	.....	.....	.....	.....	.....	3×10^{-6}	1.6×10^{-4}	0.0189

[b] Enzyme preparations Nos. 1-6 and 14 are the products of the Rohm and Haas Company of Philadelphia, Pa. According to their publication *(1)*, enzyme preparation A has a relative enzyme strength of 1, contains 4% gelatin and the remainder is dextrose; preparation B is similar to A but contains diatomaceous earth instead of dextrose; preparation M contains only dextrose as filler; preparation W has 1.5 times the enzyme activity, 25% gelatin and remainder is dextrose; the pectinase preparation was a special one. Pectinol A, M, 10M, and W-129 were lots obtained on 9/9/47; Pectinol W was a year older. Enzyme preparation No. 8 was a fungal pectinase from Takamine Laboratories, Inc. of Passaic, N. J. Enzyme preparations 7, 9, 10, 11 were fungal pectinase preparations from Wallerstein Laboratories, Inc. of New York, N. Y. Preparations 12 and 13 were from Jacques Wolf and Company of Passaic, N. J.

[c] Pectin esterase activity in the units of Lineweaver and Ballou *(21)*; activity in terms of quantity of enzyme that at 25° C. and pH 4 and in presence of 0.15 *M*. NaCl will catalyze the hydrolysis of methyl ester groups at an initial rate of 1 milliequivalent of ester bonds per minute per mg. of enzyme preparation.

[d] Pectin polygalacturonase activity in units of Jansen and MacDonnell *(17)*; activity in terms of quantity of enzyme that at 25° C. and pH 4 will catalyze the hydrolysis of pectic acid at an initial rate of 1 milliequivalent of reducing groups per minute per mg. of enzyme preparation.

[e] Ratio of PE to PG activity.

escaped the press cloth. Generally, 4 samples of enzyme-treated plus 1 of the control were measured in a given time interval, consequently all but the 11 liters of juice which were tested immediately were stored at 0° C., within one-half hour after pressing, to delay natural changes. They were withdrawn from cold storage, filtered, and then brought to approximately 25° C. immediately before viscosity measurements.

Lots of 2 liters of filtered juice were poured into gallon bottles. At exactly 2-minute intervals, following t equals 0 when the viscosity of the control was timed, a 20-g. sample of commercial enzyme which had been previously weighed in a 50-ml. beaker was dissolved in a little of the measured press juice from the bottle, poured back, and the contents of the bottle well shaken. This procedure, which takes about 1 minutes, was repeated until all of the enzyme sample had been washed into suspension.

Viscosity. A 10-ml. aliquot was pipetted off and placed in an Ostwald viscosimeter in a water bath at 25° C. Then 2 additional 15-ml. samples were placed in graduated centrifuge tubes racked in front of a light, the better to observe flocculation. These were used later for determining the volume and compactness of the sediment. This entire sampling procedure was repeated at 2-minute intervals, which time sequence was maintained throughout the experiment. The 8-minute lapse before the first reading was sufficient to bring the test solution to constant temperaure. Viscosity readings were then taken every 10 minutes until a visible flocculation was recorded.

A few spot checks were made of the change, if any, of the viscosity following flocculation (or sedimentation), but these were subject to gross inaccuracy caused by the varied density and aggregation of the sediments and in part by the enzyme carrier. This difficulty was encountered also when direct comparison was made of the viscosity values corrected only for time of flow for water, since the density and the pressure head is not constant, even for a given sample in a given time.

Color. To obtain some information on the comparative effect of the enzymes on color, the transmission of samples of the clear juice after filtration were determined in a Klett-Summerson photoelectric filter colorimeter, using 660, 540, and 420 mμ filters. All treated samples showed less absorption of light with all filters, but differences between samples were more marked with 420 mμ filter than with the 660 mμ filter.

Sediment. Samples taken previously to measure the volume of sediment were placed at 0° C. overnight to allow complete sedimentation before reading the next day. They were then centrifuged for 15 minutes at 2000 r.p.m. at 0° C., and the volumes read again. The ratio of the volumes due to sedimentation by gravity to sedimentation by centrifugation is the index of compactness. This is an important factor where the clarified juice is siphoned off the sediment, but not so important when filtration is used.

When the clarification process was complete, 2 g. of Filter-Cel was added to the remainder of the contents of the jugs (1960 ml.) and the juice separated from the sediment by vacuum filtration. The clarified juice was stored at 20° C. until required for analysis. The sediments obtained were evaporated to dryness at room temperature and weighed. Prior to analytical procedures, 50 ml. water was added to each sample and the pH adjusted to 2.5 with HCl before extracting for 1 hour at 95-100° C. The extracts were filtered while still hot, brought to volume, and stored at 0° C. between analyses.

For the uronic anhydride analysis the filtered extracts were used unaltered. Of this extract 20 ml. was used for the rapid pectin determination. From the supernatant, following hydrolysis and centrifugation as in the accepted procedure, 1.0-0.1 ml., depending on the methanol content of the sample, was used for methanol analysis. Qualitative starch, cellulose, protein tests of the extracts were routinely performed.

Clarified juice. In the analysis of the clarified juice the techniques used in the 2 series differed in the following respects. In Series I, pectic material was precipitated from 320 ml. juice with 2 volumes of 95% ethanol and centrifuged. The supernatant was concentrated in vacuo to half of the original volume (160 ml.) and a CO_2-uronide analysis performed, the results being expressed as free galacturonic acid. The precipitate was brought into solution in 40 ml. water and analyzed for uronic anhydride by the CO_2 method. In Series II, free galacturonic acid was determined colorimetrically as described below. Uronic anhydride was calculated as total uronide (CO_2 method) minus free galacturonic acid anhydride.

The uronic anhydride analyses were made from solutions rather than from dry samples, as in the original method. The substitution was 14 ml. unknown solution plus 16 ml. of concentrated HCl for 20 ml. 19% HCl plus powder.

Galacturonic acid. Galacturonic acid was determined by a modified naphthoresorcinol procedure *(9, 14, 48)*. The galacturonic acid was precipitated with strontium chloride solution. A 1 *N*. $SrCl_2$ was prepared by suspending 73.8 g. $SrCO_3$ in approximately 800 ml. water and heating to 50-60° C. Then 86 ml. of concentrated HCl was added slowly. When all of the $SrCO_3$ had gone into solution it was filtered, cooled, and diluted to 1000 ml. Then 25 ml. of apple juice (uronide value less than 0.3 meq. per ml.), plus 10 ml. $SrCl_2$ reagent adjusted to pH 7-8, were mixed with about 0.5 g. Filter-Cel and allowed to stand approximately 1 hour, then filtered with suction. The precipitate was washed with 5 ml. of $SrCl_2$ solution, and filtered to dryness. The filter cake and paper were suspended in 1 *N*. H_2SO_4, allowed to stand for 30 minutes, and filtered by gravity. Aliquots of this filtrate were used for the colorimetric analysis.

The naphthoresorcinol reagent was made by dissolving 0.25 g. in 100 ml. water, filtering, and adding one drop of concentrated HCl. This reagent was stored at 0° C. in the dark. To 2-ml. solution of the unknown containing 10 to 60 γ of galacturonic acid in 20-ml. test tubes closed with plastic paper-lined screw caps, 2 ml. of the reagent were added and the mixture brought to room temperature, then 3 ml. concentrated HCl added. The mixture was shaken and placed in a boiling water bath for exactly 2 hours, then cooled to about 20° C. with ice for 5 minutes, and 10 ml. of ethyl ether added. The solution was shaken well again and allowed to separate for 15 minutes. All steps in the procedure should be carefully timed. Then 5 ml. of the ether fraction was drawn off and its transmission was read immediately in a Klett-Summerson colorimeter using a 600 mμ filter.

The recovery values following the purification procedure ranged from 85-90%, as checked by both CO_2 analysis and the color calibration curve. This was about 10% higher than recoveries from the lead precipitate method of Harris *(14)*. It was for this reason that the strontium precipitation was preferred.

When a known concentration of galacturonic acid was added to apple juice the recovery values were slightly higher than from water. This may be due in part to the other substances in apple juice which are precipitated by strontium and help to carry down the strontium galacturonate in the precipitate. While it is possible to get higher recoveries by using more concentrated reagents, the high concentration of salts interferes with the solubility of naphthoresorcinol and the complexes it forms in non-polar solvents.

The pigment formed will not follow Beer's law when the concentration of galacturonic acid is greater than 60 γ. When dealing with unknown concentrations, samples of 2, 1, 0.5 ml. should be used, and intensity readings should be in the ratio 2 to 1 to 0.5. If not proportional, the solution should be diluted 5 or 10 times. Increasing the proportion of naphthoresorcinol will not increase the range within which Beer's law holds, but will shift the straight line portion to the region of higher galacturonic acid concentration, that is, values for small quantities will be too high.

Methanol. Methanol content was determined by the Bertrand and Silberstein *(4)* modification of the Deniges procedure. The fuchsin reagent used was prepared by dissolving 1 g. of pure crystal fuchsin in 1000 ml. warm water and adding 7-8 g. $NaHSO_3$ dissolved in 25-30 ml. water. After standing for 15-30 minutes, 18-20 ml. of concentrated HCl was added and the solution stored in the dark for several days. Then excess SO_2 was removed by applying vacuum and the reagent stored 2 days more before use. It should be colorless or a very pale straw yellow, and should be warmed for 2 minutes before use.

The solution to be analyzed was made up to 1 ml. in 25-ml. ground glass-stoppered tubes. After standing in a 35° C. water bath for 2-10 minutes, 0.3 ml. of 1% $KMnO_4$ and concentrated

H_2SO_4 (proportions 3 ml. to 0.2 ml., brought to 35° C.) were added. After exactly 5 minutes, 0.1 ml. oxalic acid was added, and then after 2 minutes, 3.6 ml. fuchsin reagent. The mixture was shaken and kept at 35° C. for 35 minutes for color development. The time intervals must be maintained exactly. Color intensity was read at 540 mμ, and the methanol content estimated from a calibration curve.

Natural PE and PG activity of apple juice. The pectic enzyme activity of the natural juice was determined, because it was thought that the small but finite changes in the viscosity of apple juice on storage at 0° C. were due to activity of the natural pectic enzymes. Because the buffering capacity of the juice interfered with PE determination by titration, the PE present was separated and determined by the Mehlitz method *(33)*.

RESULTS

Changes in relative viscosity during clarification. The changes observed in relative viscosity during clarification by the various enzyme preparations tested are shown in Tables 2 and 3. In both cases the same quantity of enzyme preparation was added, 10 g. per liter, corresponding roughly to 9 pounds per 1000 gallons of juice which was the commercial practice for the varieties of apples used. No attempt was made to compensate for the riper apples used in lot II even though it was known that juice from riper apples requires more enzyme for clarification *(49)*. The relative viscosity of the juice from the ripe Gravenstein apples decreased most rapidly in the first 30 minutes following the addition of the enzyme and then more slowly. The clarification time varied from 10 to 140 minutes, and the period of time at which flocculation first occurred varied from 10 to 80 minutes. The control required 20 hours for natural clarification and 100 minutes to reach a relative viscosity similar to that achieved in 10 minutes or less in the presence of added enzyme. The most rapid decrease in viscosity and most rapid clarification occurred with enzyme preparations 4, 5, 7, 9, and 11. The PE to PG ratios for these preparations, shown in Table 1, were 0.012, 0.001, 0.0047, 1.15, and 0.280, respectively. The slowest decrease in viscosity and slowest clarification occurred with enzyme preparations 2 and 11, having PE to PG ratios of 0.0047 and 0.28. In the activity ranges tested, the rate of decrease in viscosity and the time for clarification did not depend on the ratio of PE to PG activities or on the absolute PE or PG activities. The data in Table 3 for the juice from the mixture of over-ripe apples was similar to that reported in Table 2. The noticeable difference between the 2 lots of juice was the slower rate of decrease in viscosity of the control and that clarification due to naturally occurring enzymes was found. This was to be expected. The clarification time with this lot of juice varied from 2 minutes for preparations 4 and 13, to 180 minutes for preparation 10. Here too, neither the decrease in viscosity nor the clarification time was dependent on PE or PG activity. The PG activity and PE to PG ratio for preparations 4 and 10 were 3.6×10^{-4}, 1.4×10^{-5}, and 0.012 and 0.279, respectively. The Jacques Wolf "Hydrolase" with no detectible PE or PG activity was as efficient in the clarification of this apple juice as the "Pectinol" or "Pectinase" preparation. In general, however, preparations with low PG value were less active

TABLE 2

Changes in relative viscosity during clarification, Series I

Enzyme preparation No.	Ratio PE/PG	Clarification time	Relative viscosity after various periods of time in minutes										Final
			10	20	30	40	50	60	70	80	90	100	
		minutes											
1	.....	60	1.57	1.47	1.41	.....	1.39	1.37 [f]	.....	.....	.....	.....	1.37
2	.....	120	1.59	1.46	1.43	1.43	1.42	1.41	1.38	1.38	1.39	1.37	1.35 120 [f]
3	.....	80	1.58	1.49	1.47	1.45	1.43	1.42	1.40	1.39 [f]	.....	.....	1.39
4	.....	10	1.40 [f]	.....	.....	1.35	.....	.....	.....	.....	.....	.....	1.35
5	.....	10	1.34 [f]	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.34
6	.....	70	1.55	1.50	1.44	1.42	1.40	1.38	1.35 [f]	1.32	.....	.....	1.32
7	.....	20	1.42	1.32 [f]	.....	.....	.....	.....	.....	.....	.....	.....	1.32
8	.....	70	1.57	1.54	1.50	1.49	1.47	1.39	1.38 [f]	.....	.....	.....	1.38
9	1.2	10	1.38 [f]	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.38
10	0.28	140	granular enzyme plugs capillary										.....
11	0.28	20	1.39	1.33 [f]	.....	1.33	.....	.....	.....	.....	.....	.....	1.33
None	.....	20 hrs.	.....	1.63	.....	.....	.....	.....	.....	.....	.....	1.54	1.54

[f] Suspended matter first flocculated at this time.

TABLE 3

Changes in relative viscosity during clarification, Series II

Enzyme preparation	Clarification time	Relative viscosity at end of various periods of time in minutes									Final
		10	20	30	40	50	60	70	80	90	
	minutes										
1	90	1.58	1.53	1.50	1.47	1.45	1.43	1.41	1.40	1.38 [g]	1.38
2	120	1.65	1.62	1.62	1.47	1.44	1.42	1.40	1.39	1.38	1.36
3	90	1.46	1.39	1.37	1.35	1.35	1.34	1.34	1.33	1.33 [g]	1.33
4	2 [g]	1.60	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	<10	1.38 [g]	1.35	.....	.....	.....	.....	.....	.....	.....	.....
6	.....	insufficient enzyme									.....
7	<10	1.36 [g]	.....	.....	.....	.....	.....	.....	.....	.....	1.36
8	60	1.52	1.47	1.45	1.43	1.42	1.41 [g]	1.40	.....	.....	1.39
9	<10	1.35 [g]	1.30	.....	.....	.....	.....	.....	.....	.....	.....
10	180	granular enzyme									.....
11	<10	1.38 [g]	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	4	1.51 [g]	1.48	.....	.....	.....	.....	.....	.....	.....	.....
13	2	1.42 [g]	1.43	.....	.....	.....	.....	.....	.....	.....	.....
None	none at 5 days	1.65	1.67	1.66	1.66	.....	.....	1.63	.....	1.62	.....

[g] Suspended matter first flocculated at this time.

than those with high PG value, but variation in PG activity from [PGu] mg. values of 1.1×10^{-2} to 1.3×10^{-5} and variations in PE/PG ratios from 0.0012 to 1.15 had surprisingly little effect on clarification.

Clarity and color index of clarified juices. The transmittancy of the final clarified juices as measured in a Klett-Summerson colorimeter using various filters is shown in Table 4. The increase in transmittancy (lower reading) was proportionally greater with the 420 mμ than with the other 2 filters. With all filters, however, enzyme preparations 1 and 5 showed higher transmittancy, lower color values, and preparations 8, 10, and 11 higher color values for Gravenstein juice. The juice from the mixed over-ripe apples had higher color values with preparations 1, 8, 9, and 10, and least with 4, 5, 12, and 13. It was expected that enzyme preparations containing gelatin (1 to 5 and 14) would show greater bleaching effect on the final clarified juice owing to possible precipitation of the tannins

TABLE 4
Color index of clarified juices

Enzyme preparation	Percent transmittancy					
	Series I			Series II		
	660 mμ	540 mμ	420 mμ	660 mμ	540 mμ	420 mμ
1	18	72	410	26	110	665
2	19	80	450	22	98	540
3	17	80	450	22	98	540
4	31	88	385	18	65	447
5	26	74	360	23	89	500
6	19	95	485	....	....	....
7	18	95	495	26	110	620
8	20	95	490	31	117	700
9	16	87	480	32	115	700
10	18	96	515	27	115	700
11	17	97	530	....	....	....
12	....	....	....	17	80	490
13	....	....	....	7	60	460
None	54	200	>750	70	224	Too dense

and related coloring matter. This was true for Gravenstein juice but not true of the juice from the mixed over-ripe apples. Furthermore, a 6-fold increase in gelatin content (preparations 1 to 3 contained 4% gelatin against 25% in preparations 4 and 5) did not produce a more noticeable bleaching effect.

Quantity and composition of sediment produced during clarification. The volume and weight of the sediment produced and its uronide and methanol content is shown in Tables 5 and 6. In the Gravenstein apple juice series the volume of the sediment varied from about 3-4% for preparations 1, 2, 3, 6, 7, and 11 to about 12% in preparation 4 which contained the highest amount of gelatin. The least compact sediment was that obtained from preparations 4 and 5. The highest weight of sediment was obtained from preparations 7 and 9, and lowest from preparations 1, 2, 3, 6, and 8. That the sediment contained pectic substances is shown from its uronide value and its surprisingly high methanol content. Apparently pectinic acids of a considerable degree of methoxylation were precipitated during the course of clarification. The precipitate, however, consists only partly of uronide matter. The other constituents present, particularly polysaccharides and gums may constitute the bulk of this sediment, either in the native or partly hydrolyzed form. Reid *(42)* reported that the

TABLE 5
Weight and volume of sediment and its composition, Series I

Enzyme preparation	Dry weight	Volume of sediment in ml. per 100 ml. juice			Uronide	Methanol
		Before centrifuging	After centrifuging	Volume ratio before/after		
	mg. per 100 ml.				*mg. per 100 ml.*	*mg. per 100 ml.*
None	....	Suspension	0.03	....	232	....
1	40	3.7	1.0	3.7	11	3
2	40	3.3	0.8	4.1	12	2
3	33	3.7	0.9	4.0	5	3
4	106	11.7	1.9	6.3	22	3
5	110	10.0	1.7	6.1	22	4
6	30	3.7	1.0	3.7	5	3
7	250	3.3	1.0	3.3	11	2
8	47	4.4	1.2	3.8	5	3
9	197	5.0	4.0	1.3	....	4
10	140	6.0	1.2	5.0	5	4
11	120	3.7	1.0	3.7	10	3

TABLE 6
Weight and volume of sediment and its composition, Series II

Enzyme preparation	Dry weight	Volume ml. per 100 ml. compactness			Alcohol ppt. uronide	Methanol
		Before centrifuging	After centrifuging	Volume ratio before/after		
	mg. per 100 ml.				*mg. per 100 ml.*	*mg. per 100 ml.*
None	....	1.7 (24 hrs.)	<0.05	....	....	....
1	50	2.7	1.3	2.1	10.6	5.6
2	48	2.0	1.7	1.2	7.6	5.2
3	55	3.0	1.4	2.1	9.2	4.5
4	172	9.0	3.3	2.7	23	12.5
5	110	7.7	2.7	2.8	15.4	12.5
7	105	3.9	1.5	2.6	7.6	3.7
8	70	3.3	1.7	1.9	10.8	5.2
9	125	3.4	1.5	2.3	7.6	3.6
10	142	3.3	1.7	1.9	7.4	8.1
12	186	7.4	2.0	3.7	18.4	12.1
13	155	7.7	2.0	3.8	7.6	6.5

polysaccharidases occurring in enzyme preparations from micro-fungi were capable of hydrolyzing and depolymerizing araban, galactan, and xylan as well as polygalacturonides. The volume of sediment obtained from the juice of a mixture of late season apples was somewhat lower but its uronide and methanol content was higher.

Changes produced in clarified apple juice. The composition of the clarified juices obtained by enzyme treatment is shown in Tables 7 and 8. As a result of exposure to the various enzyme preparations, the soluble pectinic acid content decreased, but the free galacturonic acid obtained by enzymic hydrolysis increased and so did the methanol content. The tannin content decreased when enzyme preparations containing gelatin were used, particularly with preparations 4 and 5 containing 25% of gelatin. This change in tannin content was lower in Series II than in Series I.

Enzyme activity in apple juice. In view of the noticeable although slow clarification of Gravenstein apple juice in absence of added enzymes, it was thought that this juice would contain detectable amounts of pectic enzymes. To test this hypothesis the following procedure was used.

First 500 ml. of benzoated apple juice, stored at 0° C. for 1 month, was strained through a milk filter disc, then 1000 ml. of 90% ethanol was added in a very fine stream, delivering the first 500 ml. at a rate of 100 ml.

TABLE 7
Analysis of clarified juice, Series I

Enzyme preparation	Pectic acid	Free galacturonic acid	Methanol	Tannin	Tannin remaining following clarification
	mg. per 100 ml.	*mg. per 100 ml.*	*mg. per 100 ml.*	*mg. per 100 ml.*	%
None	230	65	15	56	....
1	5	80	18	....	....
2	5	102	18	35	63
3	10	88	20	53	95
4	<2 trace	93	17	27	48
5	<2	89	17	31	55
6	40	108	21	44	79
7	30	129	23	53	95
8	40	94	19	53	95
9	20	85	18	56	100
10	15	76	18	44	79
11	20	115	20	44	79

TABLE 8
Analysis of clarified juice, Series II

Enzyme preparation	Pectic acid	Free galacturonic acid	Methanol	Tannin	Tannin remaining following clarification
	mg. per 100 ml.	*mg. per 100 ml.*	*mg. per 100 ml.*	*mg. per 100 ml.*	%
None	370	52.5	17.5	87.0	....
1	80	75.0	25.2	76.5	88
2	95	67.5	24.9	80.0	92
3	120	53.5	25.4	79	91
4	5	63.5	23.2	61	70
5	5	63.5	23.4	61	70
7	50	82.5	27.8	82.5	95
8	120	65.5	26.2	79.5	92
9	20	69.0	24.8	62.5	72
10	about 5	60.0	22.2	67.0	77
12	±5	50.0	18.3	65.0	75
13	±5	56.0	20.5	60.0	69

per hour and the second portion at 5 times the original rate. The mixture was stirred constantly by an electric stirrer during the entire operation, to prevent inactivation of the enzymes by high local concentration of alcohol. This method yielded PG and PE as high in activity as by dialysis which was tried for maximum yield but which requires 4 times the time and attention.

After the concentration of alcohol reached 60%, a fine flocculation was apparent. The mixture was then stored overnight at 0° C. In a second preparation the mixture was immediately filtered. There was no appreciable difference in the activity of the two preparations, although the first was browner in color. Filter-cel (1 g.) was added to the mixture before filtration to dryness on a vacuum filter. This dry enzyme Filter-cel powder was suspended in 20 ml. water, brought to pH 4.0 with 6.0 *N*. NaOH (original pH of the enzyme-powder suspension was 3.6) and used in the following tests. This 20-ml. suspension was the equivalent of 500 ml. of apple juice. In determination of PE, 4 ml. of this enzyme preparation were mixed with 12 ml. of pectin-NaCl substrate and 4 ml. of water, to give a total volume of 20 ml. After mixing, the pH was brought to 4.0. There was no measurable change in pH after 1 hour; 2 drops of toluene were added to each beaker and samples were stored at 25° C ± 2° overnight. The control samples were treated as above, except that the enzyme preparation was heated in the oven at 100° C. for 10 minutes before addition. The changes in pH and methanol contents observed are given in Table 9.

The equivalents of methanol released, due to enzyme

TABLE 9
Pectinesterase activity in Gravenstein Apple Juice

Time of storage of enzyme-pectin substrate (hours)	pH		Methanol	
	Sample	Control	Sample	Control
			mg. per ml.	*mg. per ml.*
21	3.82	4.0	.....	.....
	3.77	4.0	.....	.....
40	3.64	3.94	0.33	0.13
120	3.45	3.90	1.46	0.42

action, is thus: 0.20 mg. per ml. in 40 hours is equal to 5.2×10^{-5} meq. per 100 ml. of apple juice per minute. This pectin esterase activity is small but significant. Pollard and Kieser *(41)* recently reported data on the pectin esterase activity of British apples. The flesh of Cox's orange pippin was found to have a pectin esterase activity approximately 1/30 of that of orange flavedo and 1/100 of that of tomato fruit.

The PG activity of this enzyme preparation from this apple juice was tested as usual with stock pectic acid. After 20 minutes of incubation at 25° C., 5-ml. aliquots each containing 2 ml. of enzyme preparation and 3 ml. of 0.8% pectic acid at pH 4 required 4.34 ml. of 0.1 *N*. $Na_2S_2O_3$, whereas 2 ml. of inactive enzyme preparation and 3 ml. 0.8% pectic acid required 4.83. According to this data, this apple juice had an appreciable PG activity, but since this is an isolated instance of PG activity in fruit tissue, too much significance cannot be attached to this result.

Commercial-scale Clarification

A comparison between the Wallerstein pectinase and Pectinol B was made on 2 different large lots of juice. Both were clarified overnight without noting the exact time of clarification.

Test made on October 19, 1949. The 3 varieties of apples, namely, Pippins, Jonathan, and Golden Delicious, were used in equal proportion for this study. They were washed in water at ordinary temperature, crushed in a hammer mill with stainless steel knives, and the juice was extracted by means of rack and cloth press, using hydraulic pressure. It was then treated in 1000-gallon wooden tanks with 9 pounds of Pectinol B and 4.5 pounds of Wallerstein Pectinase per 1000 gallons. Before use, the enzyme preparations were suspended in a few gallons of fresh juice, then added to the tanks and mixed well with a mechanical stirrer for about 5 minutes. The juice was allowed to stand overnight at room temperature (about 25° C.) for a total period of 16 hours before filtration next morning. In the morning the juice was examined for clarity, flavor, and color changes. No differences were observed between the 2 batches, and both tanks seemed to clarify equally well. The juice was then filtered separately in the usual pressure filter and pasteurized at 170-175° C. in a flash pasteurizer. It was bottled hot at about the same temperature and allowed to cool in the open air.

Test made on December 1, 1949. The same procedure was employed in this case, also, except that the apples used were of different varieties. Pippins, Romes, and Winesaps were used in the proportion of 5 to 1 to 1, respectively. No differences were observed after treatment of the juice, and the juice clarified well in both cases.

The composition of the juices clarified by the 2 enzyme preparations is shown in Table 10. The juices were similar in color value, relative viscosity, free galacturonic acid content, and residual pectic substances. Even after storage for over 2 years no precipitation of the type observed by Marshall *(30)* was noted.

TABLE 10

Composition of two commercial lots of apple juice after clarification with Pectinol B and Wallerstein Pectinase

Treatment	Brix °	Color index			Viscosity	Total uronic anhydride	Galacturonic acid monohydrate	Pectic substances (calculated) as uronic anhydride
		660 mμ	540 mμ	420 mμ				
					%	*mg. per 100*	*mg. per 100*	*mg. per 100*
Oct. 19, 1949								
4.5 lb. Pectinase per 1000 gal.	14.7	12	50	252	1.4	94	63.2	37.3
Oct. 19, 1949								
9.0 lb. Pectinol B per 1000 gal.	14.8	15	50	250	1.4	95	65.0	37
Dec. 1, 1949								
4.5 lb. Pectinase per 1000 gal.	15.0	12	50	256	1.5	73	69.1	11
Dec. 1, 1949								
9.0 lb. Pectinol B per 1000 gal.	15.0	16	56	270	1.4	72	67.0	11

SUMMARY AND CONCLUSIONS

Several commercial pectic enzymes were analyzed and their PG and PE activity determined. When selected enzymes were applied to clarification of apple juice, no relation between PG or PE activity and rate or type of clarification was noted. Some evidence is presented for the occurrence of PE and PG in apple juice.

Acknowledgment

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Selection of Sensory Testing Panels[a,b]

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The factors determining the desirability of using special procedures in the selection of sensory test panels are discussed. The application of these special procedures in the selection of test panels for the investigation of monosodium glutamate and for testing soluble coffee is demonstrated.

The concept "panel" is used in this paper in the specific sense which limits it to groups with special qualifications which are used for special purposes.

Research in connection with the development of foods for Armed Forces use at the QM Food and Container Institute poses a variety of sensory problems. Many of them involve the consumer preference approach, but others are best solved by the use of special panels. Examples of problems which require the use of panels are measurement of differences, quantitative estimate of the intensity of component flavors, analysis of complex food flavors into various sensory properties, and rapid estimation of quality. Panels may be established to deal with a certain product or product type or to handle a special type of flavor problem. Considerations of economy of time and effort make the use of elaborate selection procedures inadvisable unless a considerable amount of testing is anticipated in a given area.

Panels have been formally established for monosodium glutamate, coffee, milk, and pepper. Each one was developed to perform a certain special sensory function in relation to a general development problem *(4, 5)*. From experimental work done in connection with these 4 panels and other special purpose groups for more limited purposes a scheme of selection has evolved. It is not a rigid one. The basic approach remains constant but actual procedures may vary.

The panel member is usually required to deal analytically with a complex situation. Hence simple factors such as sensitivity to the 4 basic tastes or to various odors will only partially determine a person's value as a panel member. His level of performance in complex test situations will depend also on other factors, including rate of adaptation and recovery, memory for flavor properties, adjustment to the test situation, skill in handling flavor perceptions and, finally, the degree of his interest and motivation. The practice of selecting panels on the basis of sensitivity only, which has been often advocated, is of limited value because it takes into account only one factor *(1)*.

It would be impossible to test independently for each of the characteristics or skills which may determine achievement. Nor is it necessary. These various factors underlie a unitary skill. They may be separated analytically, but in any given sensory test most of them will operate together. Realistic test situations may be set up requiring acts of discrimination and judgment such as will be used later in definitive experiments. In such tests each factor will have its proper weight, and relative performance becomes an adequate criterion for selecting the most useful panel members.

Application of these principles to a particular selection problem requires use of test materials of the same type, and embodying the same kinds of flavor variation, that will be dealt with later. Selection tests must be closely similar to the forms to be employed later. Basic factors such as flavor memory, ability to deal logically with flavor perceptions, and general adjustment to the testing situation will be evaluated in any test. Special test conditions sometimes must be devised for others.

Since each new selection problem involves new test materials, arbitrary standards of achievement cannot be used. Relative performance determines the selection. This approach assumes that the available population will include a sufficient number of qualified people, an assumption that need not be tested since for all practical purposes one is always limited to the available population anyway.

The procedure which has been evolved has provided efficient panels and is workable from the standpoint of time and effort involved. It involves two stages: first, testing of ability to make simple discriminations of differences between samples; second, testing of ability to reproduce qualitative judgments. Candidates are eliminated in the first stage primarily because of lack of sensitivity to the flavors involved, and to some extent because of poor "flavor memory," slow recovery from stimulation, and failure to understand the test. The second stage adds the more complex requirement of ability to establish and use stable subjective criteria.

Motivation is always an important determinant of a person's value as a panel member. Interest and desire to do well predispose to sucess and both selection stages screen out some individuals who do poorly because of lack of motivation. However certain candidates who are initially selected will later lose interest and their performance will drop, but there is no reliable way of identifying these people in advance. It is advisable to run the selection tests as a matter of laboratory routine, without directing especial attention toward them, since this better assures a normal sampling of the candidates' interest as well as their abilities.

The size of the groups initially tested affects the efficiency of the ultimate panel, since the larger the number the greater the probability of finding persons of superior ability. Initial groups at the Institute have varied between 30 and 90, depending upon the importance of the problem, the amount of laboratory time

[a] This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces and has been assigned number 371 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsements of the Department of Defense.

[b] Presented at the Eleventh Annual Meeting of the IFT, New York, N. Y., June 19, 1951.

available, and to some extent on the size of the panel required.

The first stage of selection utilizes the triangle test *(2)*, although any one of several different types of discrimination tests would serve as well *(6)*. Three coded samples are presented to the observer, two are the same and the other is a variant. The observer is required to identify the latter. Test materials are prepared to obtain representative types of flavor variation in the product concerned, also to obtain a series of graded difficulty. The exact amount of flavor difference or degree of difficulty represented by each test is not important; only the limiting cases need be specified. A test represents wasted effort when it is so easy that more than about 80% of the judgments are correct. If the flavor difference between the samples is so slight that the number of correct judgments is not significantly above the chance level, it also fails to accomplish any selection.

For economy of effort, relatively easy tests are used first. After each candidate has given 2 judgments on each of 3 or 4 tests it is possible to screen out about half the group if the tests have been properly selected. Work is continued with tests of increasing difficulty until it is possible to rank the candidates on the basis of their percentages of correct judgments. All candidates should take about the same number of tests at each level of difficulty. Only the rankings near the cut-off point between those accepted for the panel and those rejected are critical.

The second stage of selection is employed when the panel is to be used for estimating quality, or for other types of tests where subjective impressions must be quantified. Approximately twice as many persons as desired on the panel are taken from the top of the rankings on the discrimination test. They are further tested in a situation involving repetition of quality judgments on a series of samples which represent a considerable range either of total quality or of some sensory property. It is important both how well the candidates reproduce their ratings and how sharply they discriminate between samples of the series. Achievement is evaluated by the ratio of between-sample variability to within-sample variability using a statistical approach which has previously been described in connection with the panel selection problem *(3)*.

When the second stage is completed 2 measures of comparative skill are available for each panel candidate. In general the top rankings will agree; however this is not always the case. Comparative weights to be given the 2 types of tests in making the final selection must be determined by the use to which the panel is to be put. One possible solution is to have, in effect, 2 panels, one for each type of study. This is not always practicable, however.

EXPERIMENTAL

Use of these procedures may be illustrated by description of the development of 2 special panels.

Selection of a panel to participate in an investigation of monosodium glutamate required only the first stage. The basic problem was to evaluate the material as a flavoring agent and the primary methodology was that of consumer preference. However, a panel was needed to measure accurately the amount of flavor difference caused by the monosodium glutamate so that only real and controlled flavor differences would be presented to the consumers. Since this would be its sole function, only the first stage was pertinent. However it was necessary to test observer skill at detecting monosodium glutamate in a number of different food types since its effect might not be the same in all.

The initial group was composed of 49 candidates, selected on the basis of availability and probable good motivation. The triangle technique was the method employed since it was to be used by the panel in the actual studies.

Each observer participated in a series of tests comprising representative food types and involving various concentrations of monosodium glutamate in the different sample.

Results of the tests are given in Table 1. The data show the wide range of skill represented in the group. In this case, a cut-off point between panel and non-panel was established at 60% correct. The 3 factors considered were: (1) the number of panel members required, (2) the precision indicated by the nature of the problem, and (3) the significance level of the differences between the individual scores and the chance score of 33⅓% on the triangle test. On the basis of the 60% correct cut-off point 25 persons were selected and 24 were rejected. A panel of 25 was adequate in size and the scores of the 3 lowest ranking members were significantly above 33⅓% at the 1% level of confidence. The intermediate group of those who scored 55% to 59% correct were selected as alternates to serve in the event any regular panel members were absent. These 6 observers all had averages above the modal tendency for the entire group (see Figure 1) and the poorest score was significantly above 33⅓% at the 5% level of confidence.

TABLE 1

Triangle test achievement of candidates for monosodium glutamate panel

Candidates in rank order	Number of tests	Percent correct	Candidates in rank order	Number of tests	Percent correct
1	17	94	26	17	59
2	20	90	27	19	58
3	10	90	28	16	56
4	14	86	29	16	56
5	20	85	30	20	55
6	22	82	31	20	55[d]
7	20	75	32	20	50
8	20	75	33	20	50
9	18	72	34	18	50
10	24	71	35	18	50
11	21	71	36	10	50
12	14	71	37	20	45
13	20	70	38	20	45
14	20	70	39	20	45
15	20	70	40	20	45
16	20	70	41	14	43
			42	14	43
17	16	69	43	12	42
18	22	68	44	20	40
19	22	68	45	14	36
20	20	65	46	12	33
21	20	65	47	20	30
22	22	64	48	20	30
23	20	60	49	20	20
24	20	60			
25	20	60[c]			

[c] Significant at the 1% level. Candidates with 60-94% correct selected for regular panel.

[d] Significant at the 5% level. Candidates scoring 55-59% correct selected as alternates.

It is of passing interest to note that the frequency polygon showing the candidates' performance (Figure 1) approximates the normal curve. This might be expected when it is considered that relative performance is dependent on physiological differences in sensory acuity, adaptation, and recovery; also on differences in such psychological factors as perception, memory, and motivation. Such biological characteristics are usually distributed normally in a population. Here of course, the normal distribution depended also on pre-selection of the tests to obtain a reasonable range of difficulty.

The second example concerns the development of a panel for testing soluble coffee. This panel was intended for 2 purposes. It would be used to determine the effect of processing variables and storage conditions, which requires difference testing, and for

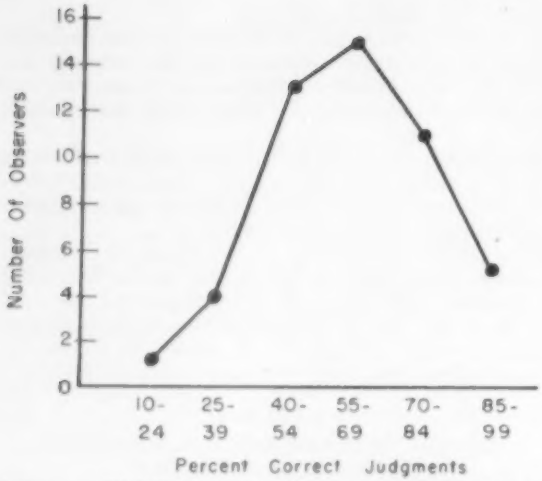

Figure 1. Distribution of observer candidates for monosodium glutamate panel according to percent correct judgments.

making rapid estimations of quality. Because of this dual function both stages of selection were needed.

Candidates were first tested for ability to detect flavor differences between samples of coffee in the same manner as the group for the monosodium glutamate panel. From an original group of 90 observers, 47 were selected on the basis of a cut-off point established at 50% correct judgments. Of the 47 candidates selected in the first stage, 38 completed the series of tests comprising the second stage.

Each candidate participated in a series of tests in which he rated the quality of coffees that required judgment in terms of degree of excellence by use of a scale whose 9 categories ranged from "extremely poor" to "extremely good." First, 9 soluble coffees and 3 freshly brewed coffees, representing a wide range of quality, were rated in pre-determined, but randomly selected groups of 3. There were 16 such tests in all so that each observer gave a total of 48 judgments, 4 replications on each sample. The data for each person were analyzed separately using the statistical technique of analysis of variance. The basic assumption in use of this technique as applied to this problem is that if an observer discriminates between samples and at the same time is consistent in his repeated judgments, the variance between samples will be considerably larger than the variance within samples. The ratio between these variances is known as the F-ratio *(7)*.

Table 2 shows the rank order of the observers according to their F-ratios, which is an index of comparative ability to reproduce judgments while discriminating between quality levels. The 27 observers whose F-ratios were higher than that required for the 5% level of confidence were chosen for the panel.

TABLE 2

F-ratios of coffee panel candidates in second stage of selection

Candidates in rank order	F-ratio	Candidates in rank order	F-ratio
1	12.49	20	2.66
2	7.80	21	2.61
3	5.18	22	2.58
4	4.95	23	2.57
5	4.91	24	2.55
6	4.91	25	2.48
7	3.97	26	2.26
8	3.53	27	2.13[†]
9	3.32		
10	3.18	28	1.98
11	3.06	29	1.95
12	3.04	30	1.92
13	2.96	31	1.72
14	2.92	32	1.57
15	2.91	33	1.26
16	2.83*	34	1.21
		35	1.18
17	2.77	36	1.11
18	2.71	37	1.01
19	2.66	38	0.77

* Significant at the 1% level.
† Significant at the 5% level. Candidates with F-ratios at or above this point selected for panel.

The data of Table 3 clearly demonstrate the superiority of the selected panels in taste difference discrimination by the triangle test. The first problem was the measurement of the flavor difference between a soluble coffee and the same coffee blended with 40% of another brand. The second problem was the measurement of the effect in sauerkraut of 0.4% monosodium glutamate. The "non-panel" groups were persons with experience in laboratory taste testing who were familiar with the triangle test, but did not include anyone who had originally been a panel candidate. In each case the percentage of correct judgments by the panel is highly significant, while the percentage attained by the non-panel group is not above the chance level. The utility of the discrimination stage of selection is clearly shown.

TABLE 3

Comparison of panel with non-panel groups on triangle difference tests

	Number of judgments	Percent correct judgments	Significance
Coffee panel	30	77	Below 0.1% level
Non-panel	29	48	Not significant
MSG panel	30	83	Below 0.1% level
Non-panel	30	40	Not significant

The superiority of the panel on tests of judged quality can not be demonstrated as clearly and quantitatively, however Table 4 does show greater variability and poorer discrimination by a non-panel group as compared to the selected coffee panel. The 4 brands of coffee were judged on total quality in 3 tests by both the coffee panel and a non-panel group using the 9-point scale described previously. The range of the means is narrower in each case for the panel group. Also better discrimination of quality is indicated in that the panel rated the best coffee higher and the poorest coffee lower.

TABLE 4

Comparison of panel with non-panel groups in judging coffee quality

Brands	Number of tests	Average of means		Range of means	
		Panel	Non-panel	Panel	Non-panel
A	3	4.10	4.20	0.70	1.15
B	3	4.33	4.70	0.30	1.15
C	3	5.77	5.32	0.95	1.00
D	3	4.45	4.47	0.65	0.80

The data of Figure 2 inductively validate the second phase of selection by demonstrating what may be accomplished with a panel so selected. Figure 2 is a control chart which shows the quality ratings established by the panel for 38 samples of soluble coffee. The mean and control limits are based upon the mean ratings obtained in initial tests on 9 major brands. There are 12 brands represented. All samples were from different production lots, with the exception of brands A and C, where tests were repeated at different times using identical materials to test the reliability of the panel. Mean ratings on coffees "A" and "C" fall within a satisfactorily narrow range while in many

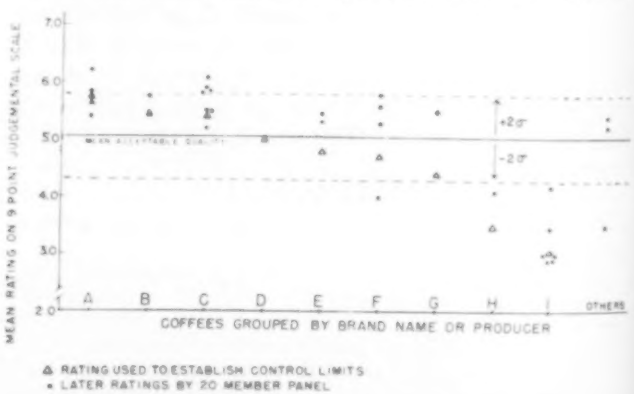

Figure 2. Control chart for soluble coffee quality.

cases wide differences were found among the samples of unknown quality.

DISCUSSION AND SUMMARY

A general approach to selection has been developed and illustrated but specific tests have not been recommended. The general approach will be of greater value than the specific examples to anyone who wishes to use the methods. It may be summarized step-wise as follows:

1. Use as test materials the same product type that will be dealt with later.
2. Prepare tests to obtain variations in the product similar to those which will be met with later.
3. Adjust the difficulty of the tests so that the group as a whole will discriminate between samples but some individuals will fail.
4. Use test forms closely similar to those to be employed later.
5. Start with as large a group of candidates as is feasible and with the selection test that is operationally simplest if more than one stage is required.
6. Screen on the basis of relative achievement, continuing until a top-ranking group of the size desired may be reliably selected.
7. Reject at each stage those persons who are obviously inadequate but carry forward more than will be required for the panel.

This procedure is not routine, but requires judgment by the experimenter, particularly as to the criteria of achievement and as to how much data are needed for valid selection. The multiple-stage selection assumes a good positive correlation between skills, but recognizes that it will not be perfect. No recommendations have been made as to the weighting of scores made in different stages. This must depend upon the particular problem.

If the assumption is valid that general ability in testing a given product can be accurately predicted from achievement in a limited number of pre-tests an even more useful generalization is suggested. It might be possible to select a "general purpose" panel using certain significant product types as test materials. Experience in selecting taste panels for four different products from among one basic group of candidates has shown that this idea has some merit. Certain individuals have been selected for all 4 panels, which might have been expected since many elements in the test situations are common. But the question of differential sensitivity to various flavors still remains. Many candidates have done well on some products but poorly on others and it is evident that any general purpose panel would be less useful for a given product than the panel actually selected. However, the "general panel" idea holds possibilities as a time-saver when precision must be sacrificed to save time and labor.

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The Use of Whey Protein in Process Cheese Production

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(Manuscript received June 26, 1951)

Some aspects of the action of calcium chloride on the heat coagulation of whey protein have been studied. By the addition of calcium chloride, an acid whey protein was obtained which blended well with hard cheese to give satisfactory process cheese and cheese spreads.

A procedure for the large scale manufacture of this product is given.

The necessity for the maximum utilization of foods is evident in Israel, where great quantities must be imported. One of the products which to date has not been fully exploited is whey, of which vast quantities go to waste each year because of the lack of economic means of utilization. The disposal of this material as waste has entailed considerable expense and inconvenience.

Whey contains much of value to nutrition, its protein being of special significance because of the pronounced deficiency of animal proteins in this country. These proteins make up 15-20% of the total protein content of milk. Their nutritional value has been estimated by Supplee and Clark *(6)* as 1.25 times that of casein and twice that of soy proteins.

The simplest method for protein removal from whey under local conditions appeared to be heat coagulation. In a recent review, Webb and Whittier *(7)* list a number of methods for the utilization of whey protein (the heat coagulable fraction as a whole) for food purposes. These methods, however, were not suitable for conditions in this country. The most practical outlet seemed to be the use of whey protein in the manufacture of process cheese and cheese spreads. We started with sweet whey because this product is concentrated in a few large dairies and the costs of its collection are comparatively small.

The simple heat coagulation of whey protein, with or without the addition of acid, or according to the method of Peter as described by Fleischman *(4)*, did not give a suitable product. When incorporated in process cheese, it did not blend properly, and the product was grainy. The same results have also been found by Burkey and Walter *(3)*. Attempts to incorporate this whey protein in white cheese (similar to cottage cheese) were unsuccessful. The work reported in this paper describes the attempts to modify the whey protein so that it would blend properly in process cheese.

LABORATORY EXPERIMENTS

Experiments were started on the influence of ions on the heat coagulation of whey protein because of their known effect on the colloidal properties of proteins. It was assumed that the modification of these properties would give the desired product. Calcium ion appeared to be the most suitable and its chloride was used, since this salt is in general use in dairies.

Materials and Methods

Fresh (whole) milk was passed through a manual cream separator. The skim milk was heated to 42° C. and coagulated with 0.2% of a fresh 2% solution of a commercial rennet preparation. The time of coagulation varied from 30 minutes to 1 hour. The curd was cut, left 15 minutes for the expulsion of the whey, and then it was separated from the whey by filtration by means of fluted filter paper (student grade).

The calcium chloride used throughout the experiments was $CaCl_2$, anhydrous, practical (Eastman Kodak).

The pH measurements were carried out with a Beckman model G pH meter. Adjustments to the desired pH were made by addition of 10% HCl or 10% NaOH after the calcium chloride had been added, because the pH usually decreased when the latter was added.

The elevated temperatures necessary were obtained by the use of an oil bath (accuracy ± 0.5° C.). Heating time was measured from the moment of temperature equilibrium.

The protein content of the whey or the filtrate obtained after coagulation was determined by the semi-micro Kjeldahl technique described by Babad, Avidor, and Shenhav *(1)*. The amount of protein precipitated was obtained by difference. Results were calculated as percentages of the original whey.

Results

Influence of pH, time of heating, and temperature on coagulation. Test tubes (12 x 100 mm., of thin glass) containing 10 ml. whey each, at various pH levels, half of which contained 0.5% added calcium chloride, were placed in the constant temperature bath. At predetermined times, after reaching the desired temperature, a number of test tubes were removed, wiped dry, and placed in an ice bath for 5 minutes. The tubes were then taken out and the relative turbidity or flocculation in the tubes was noted.

It is evident from the results in Table 1 that the calcium ion has a distinct influence on the stability of whey protein on heating. This effect varies with pH and is shown in Figure 1, which gives the lowest temperture necessary for turbidity formation in 15 minutes at the various pH levels used. Above pH 5.6 the addition of 0.5% calcium chloride increased the ease of turbidity formation, while the reverse is true below this pH. Turbidity in the absence of calcium chloride appeared at the lowest temperature at pH 4.6, which is the isoelectric point of whey protein.

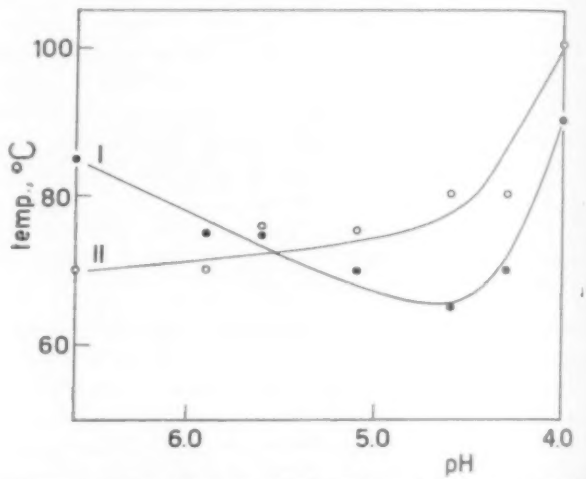

Figure 1. The effect of added calcium chloride on turbidity formation in whey by heat: The minimum temperature necessary for turbidity formation in 15 minutes is plotted against pH. ● is whey; ○ is whey plus 0.5% $CaCl_2$.

In a parallel manner cloud point determinations at pH 4.2 were made according to the method of Ballou, Boyer, Luck, and Lum *(2)*. These results are shown in Table 2. This pH especilaly interested us as shown in another part of the paper.

These results, the enhancing of the heat stability of whey protein by calcium chloride at pH 4.2, agree essentially with those described in Table 1. The differences

TABLE 1

The effect of various factors on the heat coagulation of whey protein [a]

	pH	Heating temperature																					
		65° C. Minutes of heating					70° C. Minutes of heating					75° C. Minutes of heating					80° C. Minutes of heating					85° C. Minutes of heating					90° C. Minutes of heating					100° C. Minutes of heating				
		2	4	6	8	15	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15
Whey	6.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	2	2	2	2	3	3	4	4	5	5	5	5	5
	5.9	0	0	0	0	0	0	0	0	0	1	2	3	3	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
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	4.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	2	2	3
Whey plus 0.5% $CaCl_2$	6.6	0	0	0	0	0	0	0	1	2	3	3	3	3	3	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	5.9	0	0	0	0	0	0	1	3	3	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
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	4.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2

[a] 0, no change; 1, very slight turbidity; 2, turbidity; 3, considerable turbidity; 4, flocculation; and 5, flocculation complete in 2 minutes.

TABLE 2

Cloud point of whey at pH 4.2

Temperature	Cloud point time for	
	Whey	Whey plus 0.5% $CaCl_2$
° C.	*seconds*	*seconds*
100	5	5
95	10	10
90	10	30
85	60	300
80	150	900
75	300	.....

between the 2 tables are due to the different methods used (the equilibrium in cloud point determinations is rapidly attained).

It is evident from these results that at pH values above 4 a temperature of 90° C. for 15 minutes is adequate for the precipitation of whey protein. In subsequent experiments a temperature of 92° C. was used. The time of 15 minutes was used because longer heating did not significantly increase the quantity of coagulum *(1)*.

Quantity of coagulated whey protein. Table 3 gives the quantities of whey protein precipitated with or without the addition of calcium chloride under conditions described.

TABLE 3

The influence of $CaCl_2$ on the percent of coagulated whey protein at 92° C. for 15 minutes at various pH levels

	Percent protein coagulated,[b] at pH				
	3.8	4.2	4.6	5.2	5.8
Whey	0.36	0.46	0.47	0.46	0.47
Whey plus 0.5% $CaCl_2$	0.40	0.46	0.45	0.46	0.47

[b] The average total nitrogen content of the unheated whey (in all the experiments) calculated as protein was 0.88%.

These results indicate that in the pH range investigated the calcium chloride had no significant effect on the quantity of protein precipitated. The quantity of precipitate appears to be relatively constant over the range 4.2-5.8. At pH levels less than 3.6-3.8 there was no coagulation at all.

The addition of several other ions (NH_4, Na, K, Mg, Ba) in equivalent quantities and under the same conditions, did not significantly influence the quantity of the precipitate.

Further determinations of protein coagulation made at pH 4.2 support the conclusion that 15 minutes at 92° C. was absolutely sufficient for the maximum recovery of whey protein. The results are given in Tables 4 and 5.

Settling rate. In the course of the experiments it was noted that the addition of calcium chloride altered the texture of the coagulum and its rate of settling. With reference to texture, it was observed that the coagulum was generally grainy in consistency without the addition of calcium chloride, while the addition of calcium chloride produced a coagulum that was pasty. The above results were found at all pH levels employed.

The relative settling rate was measured as follows: 250 ml. of whey at the appropriate pH, with or without the addition of calcium chloride, were placed in test tubes (45 x 260 mm.). The tubes were heated for 15 minutes at 92° C., and then cooled to room temperature and stirred. The height of the precipitate in the tubes

TABLE 4

The percent of whey protein coagulated at pH 4.2 when heated at various temperatures for 15 minutes.

	Percent protein coagulated upon heating at			
	73° C.	82° C.	87° C.	92° C.
Whey	0.21	0.31	0.45	0.46
Whey plus 0.5% $CaCl_2$	0	0	0.43	0.46

TABLE 5

The percent of whey protein coagulated at pH 4.2 when heated for various periods at 92° C.

	Percent protein coagulated upon heating for		
	5 minutes	15 minutes	30 minutes
Whey	0.46	0.47	0.47
Whey plus 0.5% $CaCl_2$	0.47	0.47	0.45

was measured when the protein without the calcium chloride had settled to the height of 80 mm. (about ½ to 1 hour after stirring). This procedure was chosen because the settling rate without calcium chloride was usually the highest. When enough time was allowed the volume of the coagulum in all cases was almost identical.

Figure 2 shows the appearance of the test tubes when varying concentrations of calcium chloride (0.1-1.0%) were added at pH 4.2. At this pH, the differences noted were most significant, and the optimum calcium chloride concentrations for producing them were 0.3 to 0.6%. It was from these experiments that we concluded that the effect of calcium chloride on the colloidal properties of whey protein, as manifested by the different settling rates, was most pronounced at pH 4.2 and 0.5% calcium chloride. As previous experiments have shown that the quantity of coagulated whey protein is independent of the presence of calcium chloride, its effect must therefore be mainly on the voluminosity of the precipitate.

Similar studies carried out at higher pH levels showed that the rate of settling decreased on the addition of

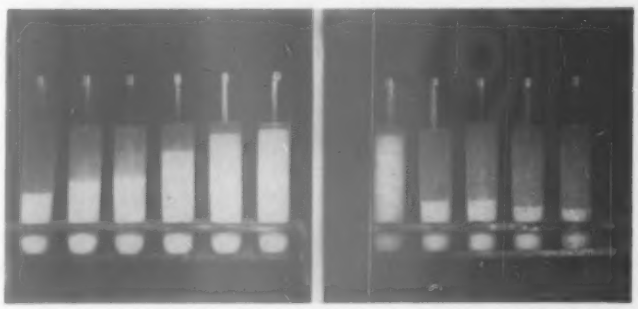

Figure 2. Demonstration of variation in settling rate of whey protein with varying concentrations of calcium chloride. The pH was 4.2 and the calcium chloride concentration varied from left to right from 0 to 1.0% with gradations of 0.1% between tubes. The photograph was taken 30 minutes after mixing at room temperature.

calcium chloride up to approximately pH 5. Measurements, however, became more and more difficult as pH levels increased, and above pH 5 it was impossible to make satisfactory determinations. Instead of settling homogeneously, the protein column separated into distinct clots, distributed throughout the liquid.

The effect of calcium chloride is evident only up to a concentration of about 0.6%, above which the differ-

ences in settling rate on its addition diminish rapidly.

Under the conditions of the experiments, reproducible results were obtained repeatedly on mixing the precipitate with the supernatant in the test tubes. The temperature had no effect on the relative settling rate.

The addition of calcium chloride after coagulation, however, did not have any influence on the properties of the whey protein.

Discussion

The experiments described have shown that the addition of calcium chloride to whey in the coagulation of whey protein by heat has the distinct effect of stabilizing certain properties of the whey protein so that at pH levels below 5.6 the coagulation temperature is increased considerably, the settling rate is decreased, and the consistency of the whey protein is improved.

Although these experiments were largely carried out in an empirical manner mainly for application to industry, the results obtained may have some theoretical significance in the coagulation of the protein aggregates. A more fundamental study of the interaction between whey protein and calcium is being planned.

The process of protein coagulation is usually considered to take place in 2 main steps: denaturation (opening up of the protein molecule), and flocculation (aggregation of the denatured molecules). It is assumed that these steps are separate and that flocculation must be preceded by denaturation.

The observed effect of calcium ion must therefore be on one of the above reactions. It seemed to us that it affected largely the first step of the reaction, that is the calcium ion protects the protein from denaturation. Some preliminary experiments in viscosity measurements and sulphydryl group determinations tend to support this assumption. The action of calcium appears to be nonspecific because qualitative experiments have shown that other ions (mono- and divalent) exerted similar effects.

The different settling rates may be due to different micellar volumes, as the viscosity of the medium in which the protein settled did not change. This increase in voluminosity may be attributed to a greater degree of hydration caused by the calcium and other ions as well. Some experiments carried out in order to determine the "bound water" content of whey protein coagulated with or without calcium chloride did not give clear results. They seem however to indicate that at pH levels between 4.0 and 5.0 the whey protein prepared in the presence of calcium chloride had a higher bound water content.

PILOT PLANT STUDIES

The results obtained from the laboratory experiments formed the basis for pilot plant studies.

Quantities of 30 to 500 liters of sweet whey, obtained from the cheese factory or prepared from skim milk as described previously, were used. Then 0.5% calcium chloride (B. P.) was added and the pH adjusted to the proper value by the addition of 1 to 1 HCl (technical). The pH measurements were made with a Beckman Model H2 pH meter or with commercial indicator paper (Lyphan). The whey was heated in a boiling water bath, or in containers with a steam jacket. Heating time was 15 minutes at 92° C. Cold water was passed through the steam jacket and the coagulum was allowed to settle. The clear supernatant was poured off and the remainder was filtered through a cheese cloth. The whey protein was allowed to drain, and then stored in a cold storage room until needed. It did not deteriorate for several weeks under these conditions.

The quantity of whey protein obtained was 3-4% of the original whey. This product had the following composition: 80-85% water, about 4% lactose, 11-14% protein, and about 1% of other constituents. A recovery of approximately 90% was obtained.

The whey protein was used for blending with hard cheese in various proportions: 20-50% whey protein, the remainder being hard cheese. Samples of about 1 kg. were prepared according to the following method: the hard cheese was ground, whey protein was added, and then emulsifying salt solution amounting to 15%, as calculated on the basis of total cheese-whey protein mixture, was added. The emulsifying solution contained 14.7% citric acid and 7% sodium bicarbonate. The mixture was well stirred under reduced pressure at a temperature of 80° C. for approximately 5 minutes. The product was packed while still warm, and cooled at room temperature. It was then stored in the cold storage room. The product obtained is used as process cheese or as cheese spreads according to whether its whey protein content is high or low, respectively.

The water content of the final product is 50-55% for process cheese and 65-70% for cheese spreads. The product is very tasty and has the desired texture. The effect of calcium chloride is shown most clearly in Figure 3. The grainy texture of the process cheese

Figure 3. The influence of added whey protein on the texture of process cheese. Calcium chloride was used in the preparation of the whey protein added to the cheese on the right. The whey protein added to the cheese on the left was prepared without any additions.

with the incorporated protein produced without the addition of calcium chloride is clearly evident. Although Okuda and Zoller *(5)* stated that the use of calcium chloride in the removal of coagulated whey protein could be discontinued, it is clear that this is not true for all cases.

In the course of the dairy experiments it became evident that for practical reasons pH 4.2 was undesirable. Most of the dairies did not have adequate pH control and it was necessary to add calculated amounts of acid to the whey. The danger of going below pH 4.2 was always present, and as there is a rapid decrease in whey protein recovery below this pH, this was to be avoided. In addition, filtration at this pH was laborious and slow. At pH levels above 5, however, the product did not blend so well with hard cheese.

By working at pH 4.6 to 5, there is a greater margin

for safety, filtration is more rapid and the product still retains its desired consistency. The quantity of acid usually added is about 200 ml. of 1 to 1 HCl to 100 liters of sweet whey. When other acids (sulfuric or acetic) were used, the same results were obtained.

Acknowledgment

The authors wish to acknowledge the assistance of Mrs. A. Shenhav-Hetman.

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The Source of Bacteria in Processing and Their Significance[a] in Frozen Vegetables

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Studies were made on a total of 5,368 samples of peas, beans, and corn during processing and after freezing storage. No pronounced relation was found between number of organisms present and quality of stored frozen vegetables. A relative constant "base" freeze-resistant flora was found in peas, beans, and corn which bore only an indirect relation to the number of organisms present prior to freezing.

INTRODUCTION

The presence of excessive numbers of organisms on food processing equipment is not a desirable condition either in canning or freezing operations. Although in canning the product generally is sterilized, the presence of high counts on equipment remains a potential cause of spoilage. When freezing is the object of the process the temperature commonly used in commercial storage, 0° F. (—17.7° C.) does not sterilize the product. Although the bacterial population may be significantly reduced during storage, a sufficient number of organisms may remain to cause spoilage when temperatures are raised.

It was the objective of these studies to determine: a), the significance of factory unit operations as a source of organisms in the processing of peas, beans, and corn in both canning and freezing and b), any possible relation between the bacterial content of these vegetables entering storage and their quality after storage at +10° F. (—12.2° C.); 0° F. (—17.7° C.); and —10° F. (—23.3° C.).

Previous Studies

Although Higley *(8)* was among the first to study effect of freezing on the bacteria in or on frozen foods, the literature prior to that time is well represented with reports on the effect of freezing, freezing and thawing, and sub-freezing temperatures on the survival of bacteria. Early in the developmental days of bacteriology, Nelsons *(14)* reported on exposures of "ferments" to —91° C. for long periods which decreased their viability but found under similar conditions that "toxins" were not destroyed. The literature on these subjects, particularly as they are concernd with foods has been reviewed by Wallace and Tanner *(26)*.

In vegetables, Lockhead and Jones *(11)* noted a gradual decrease in organisms during the first weeks of storage at 0° F. (—17.7° C.) and subsequently a less rapid decrease. According to these investigations certain species of *Micrococcus* and *Flavobacterium* were the most resistant to freezing. Sanderson *(18)* concluded that although 5% of the flora of frozen vegetables was of the *Enterococcus* type, these organisms overgrew all other organisms on slow thawing. Burton *(4)* however, stated that enterococci predominated in 376 samples of frozen vegetables and cantaloupe after one year at —4° F. (—20° C.).

Smart *(20)* suggested that soil bacteria were the most resistant on corn and probably were introduced by the corn ear worm. She concluded that blanching followed by freezing in storage at 0° F. (—17.7° C.) for 5 to 7 months reduced organisms in peas and beans by more than 95%. Tressler *(23)* believed that low bacteria content indicated careful attention to details in handling during processing and suggested 80,000 per g. as a maximum bacterial standard for frozen vegetables. Smart *(21, 22)* reported further on organisms in frozen vegetables and fruits, and stated organisms resisting were in the main soil types with no health, but with potential spoilage significance. Jones and Lochhead *(9)* in further studies confirmed earlier work on certain types of *Micrococcus* which were generally resistant to freezing at 0° F. for 16 to 30 weeks. Pederson *(16)* concluded that organisms in frozen vegetables were significant in reflecting sanitary conditions of processing equipment before freezing while Vaughn, Stadman, and Keuneman *(24)* concluded that the sanitary condition of the post blanching operations is of primary significance. Bedford *(2)* studied organisms on frozen fish

[a] Approved by the Director of the New York State Agricultural Experiment Station as Journal Paper No. 88F.

and found that at the higher temperatures of storage the decrease was more rapid than at the lower temperatures.

Gunderson and Rose *(6)* working with chicken chow mein found that emulsified fats and colloids tended to protect the bacteria in frozen chow mein but pathogenic enteric types were killed rapidly at —14° F. (—25.5° C.) during the first 5 days of storage.

In fruits, Smart *(19)* found several species of bacteria which survived —8° F. (—22.2° C.) for a year and in one instance more than 1,000,000 colonies per g. were found after a year of storage at this temperature. Berry and Diehl *(3)* found greater survival at +10° F. (—10° C.) than at —4° F. (—20° C.) while McClosky and Christopher *(12)* found from 2 to 14% survival of the *Staphylococcus aureus* and *Salmonella* types after 14 months at —0.4° F. (—18° C.). That the number of organisms decreased more rapidly at the higher subfreezing temperatures was later confirmed *(7, 10, 13, 22)*.

Nickerson *(15)* modified the Frost little plate technic and on comparison with the Petri plate method found counts to compare in the same general magnitude. Wolford *(27)* described a modification of the direct microscopic method stained by the Gram method which he indicates will differentiate between live and dead organisms and thus present a more complete sanitary history of the sample being examined.

METHODS

In general plan the study followed 2 lines, a, the determination of the source of organisms found in vegetable processing plants or more specifically the significance of various operations in contributing to the flora of the food; and b, the significance of the organisms in the frozen product as possibly related to quality and the relation of temperature of storage to survival and type of organism present.

In those instances in which the source of the organisms was the objective, samples were taken at various stages in the processing, transferred to sterile containers, chilled in ice water and examined in the laboratory within a maximum of 2 hours. Most samples were examined within an hour. Inasmuch as the unit operations, prior to the final package are essentially similar, samples to determine the source of the organisms were obtained from both canning and freezing lines. As an index of the sanitary condition of the equipment the number of organisms per gram on the vegetables has been recorded as the number of organisms on the equipment immediately prior to the source of the sample.

In the study on the relation of bacteria counts to quality of frozen vegetables, commercial samples were taken from the end of the production line, and placed in the factory cold room at 0° F. (—17.7° C.). If an examination on the third day indicated that the count was an addition to the graduated series of counts desired, duplicates were transferred to the temperatures of the investigation, that is, +10° F. (—12.2° C.), 0° F. (—17.7° C.), and —10° F. (—23.3° C.). The samples were selected for storage studies to give a graduated series of total counts. Additional samples, if not immediately examined, were held at —40° F. (—40° C.) and examined within 2 weeks. A total of 18 samples were examined in each case.

In determining the number of bacteria in peas and beans in all samples, rinse counts unless otherwise noted, were made in 100 ml. sterile water blanks, which were shaken vigorously. Direct weighed samples of suspended creamed style corn were used.

Tryptone-glucose-yeast extract agar was used and incubation was at 89.6° F. (32° C.) for 48 hours. Duplicate plates were incubated at 131° F. (55° C.) to determine presence of thermophiles. The accepted methods for the identification of the organisms were used *(5)*.

The stored samples at the 3 temperature levels were examined at intervals for the number of organisms present; by a taste panel for flavor, texture, and color; and the ascorbic acid, thiamin, riboflavin, and carotene content. The percent of total solids was also determined.

Ascorbic acid was determined by the xylene extraction procedure *(17)*, thiamin by the thiochrome technic *(1)*, carotene by the chromatograph technic *(22)* and riboflavin by the fluorometric method *(1)*.

RESULTS

The data were collected over a period of 4 years and involved the collection of 5,368 samples. Not all of these samples were subjected to the complete series of tests as an observation of selected samples resulted in adequate data to warrant, in some instances, a final conclusion.

Peas

A total of 2,361 samples of peas was collected for the study of the source of organisms on processing equipment, and the relation of flora to quality of frozen peas after storage. Samples were obtained from 5 factories.

One plant (C44) operated both freezing and canning lines, using the higher grade peas for freezing. The condition of the equipment was far above average. The viner was immediately adjacent to the plant and the peas conveyed immediately to the blancher and cooler with less than 15 minutes between vining and blanching. The peas were transferred by flumes through a rock remover, brine grader to sorting belt and finally by metal baskets to the filler. The second plant (W) confined operations to canning and the sanitary condition of the equipment was not of the best. Although the viner was operated at the plant, considerable time elapsed between vining and blanching and the entire operation was not carefully controlled, particularly with regard to sanitation. The third plant (GP) also confined its operations to canning. The peas were vined at 2 viners located several miles from the plant, were iced and transported within 3 hours to the plant. This plant eliminated the usual sorting belt operations substituting an additional quality brine grader. The 2 other plants (A-B) confined operations to freezing. The equipment was maintained in good condition, the vining operations were not in connection with the plant, but the vined peas were transported iced during a period of 1 to 3 hours.

Samples were secured from all of the plants in the study of the source of organisms in processed peas and only from Plants A and B in the studies on the relation of bacteria counts of frozen peas to quality after storage.

The viner as a source of organisms. Samples of peas were removed aseptically from the pods of peas arriving at a viner and in all instances the peas so removed from the pod were essentially sterile, both by rinse counts and on samples disintegrated in the Waring blendor. The aseptically removed peas were not broken so as might be anticipated there was little difference between the rinse and disintegrated counts. Comparable peas from the same loads after vining had a count of 1,300,000 per g.

To determine if any treatment following the viner operations might reduce the number of organisms in the vined peas, a series of 6 samples were observed at a viner and duplicates of these samples were treated with water containing 100 p.p.m. of chlorine and a quaternary ammonium compound (alkyl-dimethyl-benzyl-ammonium chloride) in water at 1 to 5,000 concentration. In the latter case following such treatment the peas were thoroughly rinsed with water before being observed over the period under study. The peas from these viners were held for a total of 6 hours.

The results shown in Table 1 indicated one rather significant fact, namely, that at the end of 2 hours there

TABLE 1

The effect of sanitizing agents in reducing plate counts on peas in the interval between the vining and processing operations

Series	Treatment	Number of organisms, in thousands at end of (hours)					Center temperature (° F.) at	
		0	0.1	2	4	6	Beginning	End
A	None	720	....	150	700	1,500	72	78
	None	1,040	....	200	1,150	2,260	72	78
B	None	610	....	320	420	1,310	54	81
	None	440	....	370	390	970	54	81
	Quaternary 1:5,000	610	6	13	78	8,920	66	85
	Quaternary 1:5,000	440	4	13	122	9,240	66	85
C	None	55,600	....	1,900	2,700	4,800	70	86
	None	45,200	....	2,500	2,600	4,400	70	86
	100 p.p.m. chlorine	55,600	230	5,120	340	15,300	66	84
	100 p.p.m. chlorine	45,200	160	2,760	330	13,000	66	84
D	None	15,900	....	16,500	32,000	103,000	62	85
	None	17,000	....	10,000	20,000	107,000	62	85
	Quaternary 1:5,000	15,900	58	195	440	2,130	66	83
	Quaternary 1:5,000	17,000	65	179	720	1,120	66	83
E	None	3,600	....	3,000	7,300	149,000	66	89
	None	2,800	....	2,300	7,000	110,000	66	89
	100 p.p.m. chlorine	3,600	47	123	560	3,500	67	84
	100 p.p.m. chlorine	2,800	32	118	680	4,600	67	84

appeared to be a noticeable reduction in count, particularly in the untreated samples. At the end of the 4 hour period the bacterial population showed little or no increase and at the end of 6 hours the increase was appreciable.

In the treatment with the quaternary ammonium rinse the number of organisms was materially reduced at the end of approximately 5 minutes and this reduction was still apparent at the end of 2 hours. However, at the end of 6 hours the number of organisms in the peas originally rinsed with the quaternary ammonium compounds in 1 instance was higher than the controls which had been untreated.

During these observation periods, the center temperature of the buckets of peas being observed during the 6 hour period generally increased by 6 to over 20° F. degrees.

It should be concluded from these observations that the viner is a significant source of organisms. Icing of peas is necessary and it is somewhat doubtful if a chlorine or quaternary ammonium compound rinse is a significant factor in controlling the organisms present if the peas are to be held for periods greater than 4 hours.

Pea processing equipment as a source of organisms. The significance of operating equipment as a principal source of organisms has been appreciated throughout the food processing industry. In the 5 plants, samples were obtained from strategic stations in the operations to determine the significance of the various types of equipment in contributing appreciable numbers of gas producing and other types of organisms to the peas.

Considerable variation between different plants was apparent but not dependent entirely on the visible conditions of the equipment as the visible appearance of the equipment, from the standpoint of cleanliness, could not be directly related to the number of bacteria present. In one plant (A), as shown in Table 2, the equipment was visibly clean and appeared in excellent condition, however, large numbers of both thermophilic and non-thermophilic organisms were present. Of particular interest in this plant, which is a typical operation, was the fact that the counts increased immediately following the blanching. A similar observation of rapid increase of count after blanching was made in a second plant (W) in which the equipment was not clean and the general sanitation conditions were of a low grade, and in addition, harbored large numbers of organisms. However, in this particular operation the average count was somewhat lower than in plant in which the equipment had a visible appearance of cleanliness.

It was noted that the count generally built up prior to the blancher at which point there was a definite

TABLE 2

The number of organisms per gram on peas during 6 days' sampling after passing through various unit operations at plant A

Source of sample	Number of samples	Plate count, incubated at 89.6° F. (32° C.)			Plate count, incubated at 131° F. (55° C.)		
		Highest count	Lowest count	Average count	Highest count	Lowest count	Average count
Hopper before dry cleaner	5	190,000,000	190,000	38,000,000	10,000	90	4,618
After dry cleaner	4	84,000,000	400,000	20,000,000	10,000	60	2,812
Flume before washer	4	13,000,000	160,000	3,800,000	12,000	10	4,402
After washer	5	7,800,000	24,000	2,600,000	10,000	0	2,202
After belt to blancher	5	4,100,000	20,000	1,800,000	10,000	6	2,201
After blancher	4	3,000	0	1,000	20,000	2	8,000
Flume after cooling reel	5	84,000	1,000	20,000	3,000	7	151
After cooling reel	5	23,000,000	1,000	3,800,000	30,000	3	7,500
After belt and quality separator	6	680,000	17,000	300,000	10,000	1	4,000
After quality separator	6	230,000	4,000	68,000	1,000	3	200
After flume and reel	6	70,000	2,000	34,000	3,000	4	600
After picking belt	6	310,000	4,000	140,000	10,000	0	2,000
After flume from belt	5	150,000	9,000	63,000	1	1	0.2
After conveyor belt	5	97,000	10,000	40,000	3	3	0.6
After flume and reel to hopper	6	390,000	6,000	90,000	1,000	10	402
After filler hopper	6	940,000	11,000	260,000	1,000	0	200
From filled package	6	920,000	16,000	340,000	3	3	0.6
Frozen sample	6	770,000	10,000	220,000	2	2	0.4

reduction even in the thermophilic types. However, following the blancher the count may again rise due to lack of attention to the detail of equipment, particularly the filler hoppers or other operations, if the blancher is too far removed operationwise from the final package.

Not only was the total number of organisms which may build up reduced materially by blanching, but, as can be noted in Table 3, the presence of gas-producing types followed a similar pattern. It was found in one plant (A) which was maintained in good visible sanitary condition that the presence of these gas-producing types was rather widespread through the plant with the exception of just subsequent to blanching. The processing, however, immediately up to and including the final packaging was sufficiently contaminated to contribute significant numbers of organisms to the final product.

These results indicate that by careful attention to detail it should be possible to reduce the counts to a reasonable level. No doubt particular precautions would be required to eliminate gas-producing types, particularly after blanching. It was noted in one plant (A) that the lowest count observed over a period of 12 days was generally less than 1,000 organisms per g. and the highest count generally did not exceed 100,000 per g. This possibility of maintaining relatively low counts was also observed in a second plant (B) in which samples were secured twice a day over a period of 13 consecutive days, which included practically the entire pea processing season. In this operation the maximum count prior to blanching did not exceed 600,000 per g. while the average count ranged from 29,000 to 93,000 per g.

Effect of freezing storage on organisms in frozen peas. From the above data and that of other observers, it is indicated that the factory and viner operations are the principal sources of organisms in peas to be canned or frozen. To determine if these organisms commonly originating on processing equipment were resistant to sub-freezing temperatures, a series of samples of frozen peas were obtained from plants A and B for storage observations. The 67 samples studied had a graduated count from 8,900 to 870,000 per g. on entering storage.

These 67 samples were divided into 3 arbitrary groups, the first group as shown in Figure 1 having a count at the beginning of storage of less than 40,000 per g., the second group from 40,000 to 100,000 per g., and the third group from 100,000 to 870,000 per g. It

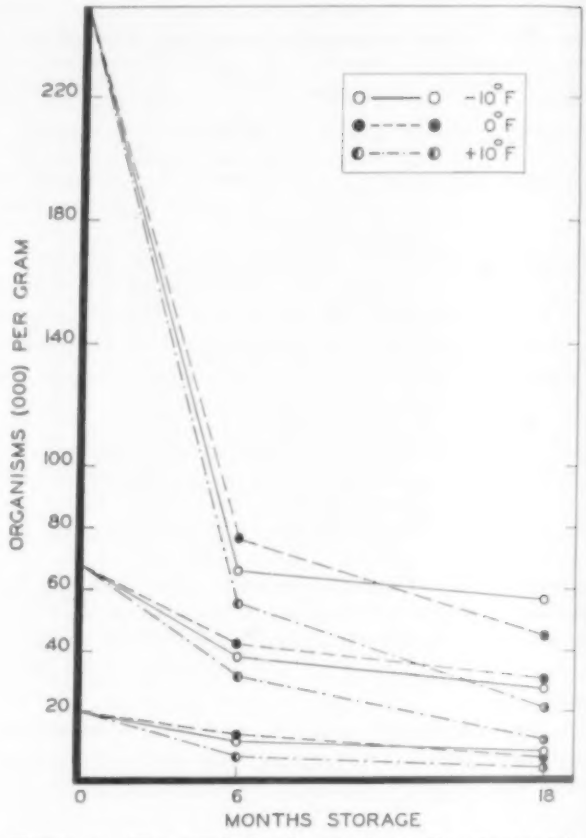

Figure 1. The survival of organisms (in thousands) in stored frozen peas. Average count of three groups of samples.

was noted after six months of storage that all 3 of these groups showed a marked reduction in bacteria count. The greatest relative reduction was in the samples stored at the highest temperature, +10° F. (—12.2° C.) and the least reduction in the duplicate series of samples stored at 0° F. (—17.7° C.). These results confirm the conclusion of Haines *(7)*, Smart *(19)*, and others.

At the end of 14 months this same relation was not altogether maintained. In the group of samples with less than 40,000 per g., the greatest reduction occurred at the highest storage temperature. This relationship was also evident, but not so striking, in the group of samples ranging in count from 40,000 to 100,000 per g. However, in the group of samples with counts greater

TABLE 3

The presence of gas-producing organisms[a] on peas after passing through various unit operations in plant A

Source of sample	Amount of gas (percent) produced by one gram sample					
	Series 1	Series 2	Series 3	Series 4	Series 5	Series 6
Flume after washer	35	22	....	46	22	25
After washer	15	20	....	40	35	31
After conveyor belt to blancher	25	15	50	20	32	35
After blancher	....	....	....	....	....	....
Flume before cooling reel	....	....	....	....	....	....
After cooling reel	33	25	....	68	9	17
After conveyor belt to quality grader	33	20	40	30	34	45
After quality grader	47	47	40	32	42	34
After flume and reel	5	40	....	45	trace	4
After picking belt	33	23	trace	38	38	25
After flume from picking belt	35	11	....	48	53	41
After conveyor belt	20	11	....	36	25	35
After flume and reel to hopper	15	5	45	20	35	28
After filler hopper	25	40	45	60	44	48
From filled package	40	11	40	58	16	40
From frozen sample	55	33	40	83	50	25

[a] Cultured in brilliant green bile broth medium in Durham tubes. Samples collected on separate days.

than 100,000 per g. the greatest reduction occurred at the 0° F. (—17.7° C.) temperature after 14 months of storage.

Although a relation was apparent between original count and the number of organisms surviving after storage, the reduction increased as original count increased. There was some relation between the number of organisms surviving at the end of 6 and 14 months storage at the 3 temperatures, but it was obvious that there appeared to be in all samples a certain base resistant flora present bearing some relation to the initial count, but with a tendency to be common to all samples.

If a bacteriological standard with a maximum of 50,000 per g. were to be considered as a standard for frozen peas, counts above this figure for peas entering storage would be of particular significance to the processor. Of the 67 sample series, 29 were below this figure when placed in storage.

A study of the 38 samples of peas ranging in count from 50,000 to 870,000 per g., as indicated in Table 4,

TABLE 4

Effect of storage on number of bacteria in 38 samples of peas with initial counts of greater than 50,000 organisms per gram

Period of storage	Temperature of storage	Samples under 40,000 organisms per g.
months		%
6	+10° F. (—12.2° C.)	73
14	+10° F. (—12.2° C.)	98
6	0° F. (—22.2° C.)	44
14	0° F. (—22.2° C.)	72
6	—10° F. (—23.3° C.)	39
15	—10° F. (—23.3° C.)	66

showed that at the end of 14 months 98% of the samples had counts of less than 40,000 per g. when held during this period of time at +10° F. (—12.2° C.). Similar samples held at 0° F. (—17.7° C.) and —10° F. (—23.3° C.) showed respectively, 72 and 66% of the samples with counts under 40,000 organisms per g.

In light of the reduction of the counts during storage, if a processor packed the entire series of 67 samples with a count up to 870,000 per g., the pack would come from the 0° F. (—17.7° C.) storage after six months with 70%, and at the end of 14 months with over 80% less than 40,000 organisms per g.

Comparable results were indicated in the effect of storage temperature on gas-producing types. As shown in Table 5, there appeared to be greater survival of gas-producing types during storage at 0° F. (—17.7° C.) and —10° F. (—23.3° C.) than at +10° F. (—12.2° C.). It is of interest to note also the large total incidence of the presence of these gas-producing organisms in the final frozen product.

Relation of initial count to quality of stored frozen peas. A selected series of 13 stored frozen samples with an initial count of less than 50,000 per g. and similar series with an initial count greater than 50,000 per g. were submitted to a taste panel for flavor, texture, and color evaluation.

At the end of six months of storage, at 0° F. (—17.7° C.) there was no appreciable change in the flavor score of any of the samples so stored. After 18 months at 0° F. (—17.7° C.) the stored samples with

TABLE 5

Percent of samples of peas showing gas-producing organisms [b] after freezing storage

Number of samples	Period of storage	Temperature of storage	Samples producing gas
	months		%
61	14	+10° F. (—12.2° C.)	29
60	18	0° F. (—17.7° C.)	99
65	15	—10° F. (—23.3° C.)	76

[b] Cultured in lactose broth.

the initial counts of greater than 50,000 per g. showed a tendency to be inferior in flavor as compared with samples of an initial count of less than 50,000 per g. The same observation was true at the end of 18 months for the limited series of samples stored at —10° F. (—23.3° C.). It is not possible to conclude that these results are of great significance as the observed series was limited. However, it is doubtful if there is a significant relation between flavor after commercial storage and the initial count on the sample if this count is less than 800,000 per g.

Ascorbic acid, carotene, thiamin, and riboflavin determinations on a series of 8 samples selected from the group with less than 50,000 per g. as an initial count, and from the group with more than 300,000 per g. for the initial count, indicated no apparent relationship between the initial count and the vitamin retention over a period of 12 months at the 3 storage temperatures used. However, there was a relationship between ascorbic retention in the frozen peas and the storage temperatures at which the peas were held. This confirms the work of Tressler *(23)*.

Types of organisms. At the end of 6 months and 14 months of storage, 174 strains of organisms were isolated from the peas. The isolations were, in most instances, from 0° F. (—17.7° C.) storage temperature. In Table 6 it can be noted that *Micrococcus* sp., *Flavobacterium*

TABLE 6

The types of organisms found in frozen peas, beans and corn after storage for 6 months at 0° F. (—17.7° C.)

Source	Total strains	Strains isolated			
		Micrococcus sp.	Flavobacterium sp. Achromobacter sp.	Streptococcus sp.	Miscellaneous
		%	%	%	%
Peas	174	24	27	22	27
Beans	53	54	13	20	13
Corn	76	56	6	26	12

and *Achromobacterium* sp., and *Streptococcus* sp., were the predominant types that resisted freezing storage temperatures.

The micrococci were generally yellow pigmented of both gelatin liquefying and non-liquefying types. A majority of these strains was of a paired diplococcoid type. Of the *Streptococcus* strains, 38 of the 39 isolated were facultative anaerobes and were generally gelatin liquefying and active in acid production and showed no peptonization of litmus milk. The *Flavobacterium* and *Achromobacterium* strains generally did not reduce nitrates, or liquefy gelatin but showed active peptonization of litmus milk.

Beans

As in the case of peas, data on beans were collected in 2 separate series. The first was concerned with the source of organisms gaining entrance to beans during the course of factory processing, and the second on the possible significance of such organisms on the storage qualities of frozen beans. A total of 1,986 samples were collected from 6 bean processing plants. Of these 4 confined their operations to freezing and 2 to canning.

Bean processing equipment as a source of organisms. In the processing of beans, prior to canning or freezing, the number of organisms on equipment appeared to be relatively greater than on pea processing equipment. This was true even in the early stages of the operations. In addition, the visible sanitary condition of the plants appeared to be more closely related to the number of mesophilic organisms present than in the case of peas. Two plants, A and B, were maintained in good visible sanitary condition in contrast to two other plants, GP and W. In one of the latter, the average counts after the cutters were as high as 85,000,000 organisms per g. As in the case of peas, there appeared to be little relation between sanitary condition as indicated by the visible cleanliness of the equipment and the number of thermophilic organisms present. The lowest incidence of thermophilic count was not in any instances, associated with the best sanitary conditions.

Beans entering the plant generally carried more organisms than peas. Peas when removed from the pods were essentially sterile while snap beans may have been subjected to contaminating contacts. During the early operations the cutters, either as a result of unsatisfactory sanitary conditions or by dispersing the bacteria and breaking up the clumps, materially increased the counts. The number of bacteria was obviously reduced by the blancher but the recontamination following blanching appeared to be more pronounced in beans than in the case of peas. This was especially noticeable in 3 plants, one of which was indicated in Table 7 showed a significant buildup of organisms, over a period of days of operation.

Of particular significance as a source of contamination were the sorting belts which appeared to be a more significant source of organisms in bean than in pea operations. In one instance, shown in Table 8, with good cleaning every 2 hours, the organisms gradually built up during the day. The counts on this particular belt over a 4-square-inch area increased to 264,000,000 organisms. This figure may not reflect the total count but is the number removed by the swab procedure. Obviously the beans did not remove an appreciable number of the organisms, although the excessive flora on the belt remained as a potential source of contamination. It should be noted that after cleaning, the count increased. This is often encountered in certain washing operations, unless followed by sterilizing procedures.

TABLE 8

Sorting belt as a source of organisms in the processing of beans

Time and condition		Number of organisms, per 4 square inches of belt surface
6:30 A.M.	Before cleaning	70,000,000
	After cleaning	224,000,000
	Water rinse	7,000,000
8:15 A.M.	Before cleaning	9,000,000
	Before cleaning	370,000
10:30 A.M.	Before cleaning	27,000,000
	Beans entering belt	4,000 [a]
	Beans leaving belt	360,000 [a]
12:00 Noon	Before cleaning	36,000,000
	Beans entering belt	8,500 [a]
	Beans leaving belt	900,000 [a]
2:00 P.M.	Before cleaning	320,000,000
	Beans entering belt	1,700 [a]
	Beans leaving belt	230,000 [a]
3:00 P.M.	Before cleaning	264,000,000
	Beans entering belt	23,000 [a]
	Beans leaving belt	1,120,000 [a]

[a] Microorganisms per gram of beans.

Effect of freezing storage on organisms on beans. To determine the effect of commercial freezing on the organisms present on beans, samples were obtained from plants A and B. The 82 samples of beans with an original count ranging from 2,000 to 2,500,000 organisms per g. were stored for 12 months at +10° F. (—12.2° C.), 0° F. (—17.7° C.) and —10° F. (—23.3° C.). These 82 samples, for comparative observations, were divided into 3 groups, that is those with 100,000 or less, with 100,000 to 310,000, and with 350,000 to 2,540,000 organisms per g. At the end of 6 months of storage the number of organisms surviving in each of these groups as shown in Figure 2, was generally in proportion to the initial count of the beans prior to storage. Again it was noted at the end of six months that the greatest reduction, as in the case of peas, was in the samples stored at +10° F. (—12.2° C.) in contrast to duplicate samples at 0° F. (—17.7° C.) and —10° F. (—23.3° C.). The reduction in organisms as shown in Figure 2 was more pronounced in the higher than in the lower count groups. Again, as in the case of peas, there appeared to be a base cold-resistant flora somewhat independent of the total initial count.

If a maximum of 100,000 organisms per g. were to be adopted as a bacteriological standard for frozen beans there is an indication that a substantial number of beans with a high initial count might meet such a standard after storage at 0° F. (—17.7° C.). Of 44 samples

TABLE 7

The number of organisms per gram, on beans during 9 days' sampling, after passing through various unit operations in plant A

Source of sample	Number of samples	Plate count, incubated at 89.6° F. (32° C.)			Plate count, incubated at 131° F. (55° C.)		
		Highest count	Lowest count	Average count	Highest count	Lowest count	Average count
Before cutter	17	4,180,000	51,000	740,000	1,000	30	322
After cutter and shaker	17	6,400,000	7,000	573,000	400	0	248
After blancher	14	1,000	1,000	1,000	210	0	75
After shaker and before sorting belt	17	3,028,000	1,000	188,000	220	0	85
Final package	17	340,000	1,000	36,000	240	0	121

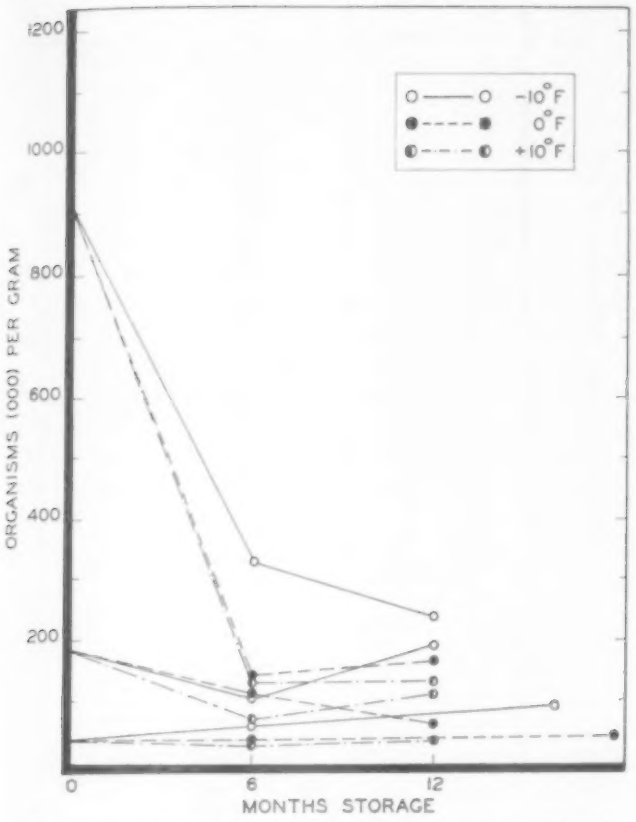

Figure 2. The survival of organisms (in thousands) in stored frozen beans. Average count of three groups of samples.

with an initial count in excess of 100,000 organisms per g., 85% were less than 90,000 per g. after 18 months of storage and 41% were below 90,000 per g. after 6 months of storage at 0° F. (—17.7° C.). It should be noted in Table 9, that the greatest reduction in or-

TABLE 9

Effect of storage on number of bacteria in 44 samples of beans with initial counts of greater than 100,000 organisms per gram

Period of storage	Temperature of storage	Samples under 90,000 organisms per g.
months		%
6	+10° F. (—12.2° C.)	65
12	+10° F. (—12.2° C.)	67
6	0° F. (—17.7° C.)	41
18	0° F. (—17.7° C.)	65
6	—10° F. (—23.3° C.)	34
13	—10° F. (—23.3° C.)	44

ganisms occurred in the +10° F. (—12.2° C.) sample in contrast to 0° F. (—17.7° C.), and —10° F. (—23.3° C.) storage.

During the processing observations on beans, only 3 complete series of samples were observed to determine the presence of gas-producing types. The results were essentially similar to those observed in peas. The gas-producing types were eliminated by blanching but the equipment became easily reinfected between blanching and the final package. These gas-producing organisms, as may be seen in Table 10, survived in beans as in peas during freezing storage and a greater survival was indicated in the lower temperatures.

TABLE 10

Percent of samples of beans showing gas producing organisms after freezing storage [4]

Number of samples	Period of storage	Temperature of storage	Samples producing gas
	months		%
76	12	+10° F. (—12.2° C.)	30
70	18	0° F. (—17.7° C.)	85
78	15	—10° F. (—23.3° C.)	75

[4] Cultured in lactose broth medium.

Relation of initial count in quality of stored frozen beans. Determinations of ascorbic acid, carotene, thiamine, and riboflavin were made on a series of 4 samples with an original count of less than 100,000 per g. and a similar series with more than 300,000 organisms per g., after storage 1 year at +10° F. (—12.2° C.), 0° F. (—17.7° C.), and —10° F. (—23.3° C.). There was no direct or indirect correlations noted between original count and quality of the stored beans as reflected by any change in vitamin content. As in the case of peas, the samples held at +10° F. (—12.2° C.) showed a greater loss of vitamins, particularly ascorbic acid, than the samples stored at 0° F. (—17.7° C.), and —10° F. (—23.3° C.).

A similar series of 24 frozen samples were submitted to the taste panel for flavor, texture, and color determinations. Beans with an original count of 100,000 or less organisms per g. were found to be slightly superior in flavor after a storage at 0° F. (—17.7° C.) for 6 months. However, in the case of color, beans with an original count greater than 100,000 organisms per g. tended to lose color on storage to greater degree than beans with an original count of less than 100,000 organisms per g.

Type of organisms present in frozen beans. As shown in Table 6, 53 isolations were made from beans, both at the 6 and 18 months of storage period of 0° F. (—17.7° C.). The flora consisted largely of strains of *Micrococcus* sp., *Flavobacterium* sp., and *Streptococcus* sp. The latter was largely of the *Enterococcus* type. Although it may not be significant, a larger percent of strains of micrococci were found in beans than in peas.

Corn

A total of 1,021 samples of corn was collected to determine, as in the case of peas and beans, the source of organisms gaining access to the corn during processing and also to correlate, if possible, the relation of the total count with quality subsequent to storage.

Corn processing equipment as a source of organisms. Samples from 2 plants, GP and B, processing corn were studied with respect to the number of organisms on the equipment. Plant GP was not operated in a high degree of sanitation, while Plant B was maintained in good condition with equipment washed and cleaned at regular intervals.

The maintenance of acceptable sanitary conditions is made more difficult in corn operations than with peas and beans, particularly where creamed style corn is being handled. Although the number of organisms gradually increased during the progress of the corn through the equipment prior to blanching, no particular operation appeared to be consistently an offender as an outstanding source of bacteria. In corn, more than in peas or beans, blanching is of particular significance in

reducing the mesophilic types, thus the condition of the equipment subsequent to blanching is even more important. In corn operations, a plant may become greatly contaminated and if operations subsequent to blanching are not carefully controlled, excessive numbers of organisms may build up rapidly. On the other hand, as noted in Table 11, if the conveyor and filler hopper are kept in good condition after blanching, it is possible to maintain a low mesophilic count in the final package.

In the case of the thermophilic types, the number present may not be closely correlated with the total mesophilic count or with the general sanitary condition of the plant. In the plant in which the sanitary condition of the equipment was the more closely controlled and the total mesophilic count remained at a relatively low level, the thermophilic count was the higher. It was apparent that the visible sanitary condition of the equipment may not be a significant factor in the source of theormophils. In the plant in which the thermophilic count remained at a low level, the thermophiles appeared not to be entering the plant as the number of organisms present prior to husking, was at a minimum. No information is available as to the number of organisms entering the second plant but shortly subsequent to the husking large numbers were demonstrable and remained throughout the process. It has been pointed out earlier by Smart *(19)* that resistant soil organisms may enter the plant on the corn as a result of an infestation of corn ear worm.

As in the case of peas and beans, the gas-producing types were found to be common throughout the processing operations with the blancher eliminating these types for a short period while they reappeared subsequently and remained to the final frozen package. As in peas and beans, the equipment is an important source of bacteria in the processing of corn.

Effect of freezing storage on counts in corn. To determine the significance of these organisms, 45 samples of corn varying in count from 51,000 to 3,620,000 organisms per g. were stored for 12 to 18 months at +10° F. (—12.2° C.), 0° F. (—17.7° C.), and —10° F. (—23.3° C.).

To facilitate evaluating the effects of storage on organisms on corn, samples were assembled into 3 groups, one with counts of 81,000 to 160,000 organisms, another with 160,000 to 1,000,000 organisms per g., and a third with 1,000,000 to 3,500,000 organisms per g. The most pronounced reduction in count, as shown in Figure 3, was after 6 months of storage at +10° F. (—12.2° C.) In addition, corn as well as peas and beans, appeared to have a base flora resistant to freezing.

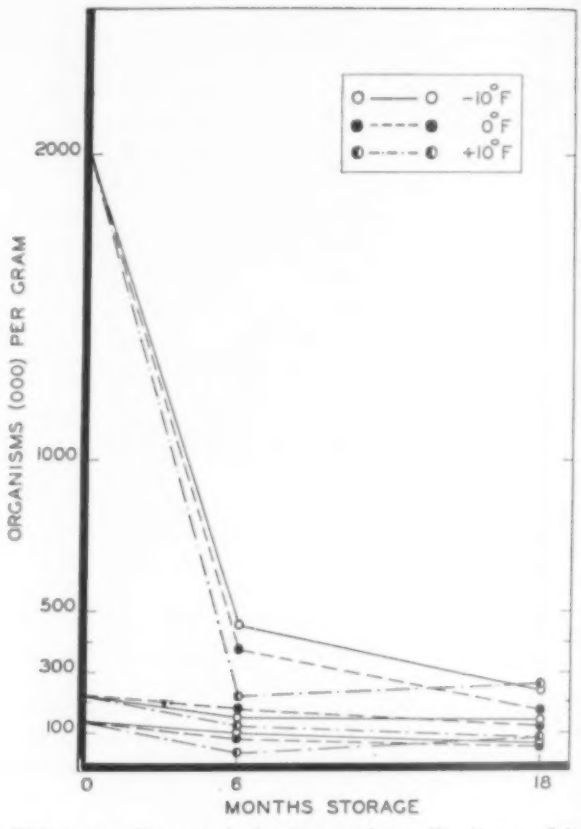

Figure 3. The survival of organisms (in thousands) in stored frozen corn. Average count of three groups of samples.

Although bearing some relation numerically to the initial flora of the corn, the average reduction in count during storage at 0° F. was more pronounced in the higher than in the lower count corn. The 10 samples with an average count of approximately 2,000,000 organisms per g., showed an average of 377,000 per g. and 173,000 organisms per g. after 6 and 12 months of storage at 0° F. (—17.7° C.), respectively. The 35 samples with less than 1,000,000 organisms per g. under similar storage conditions show a less appreciable reduction.

The average count is materially reduced, the incidence of samples showing reduction, although significant, is not as appreciable as in the case of peas and beans. The greatest incidence of reduction was in the samples stored at +10° F. (—12.2° C.). At the usual storage temperature of 0° F. (—17.7° C.) however, 62% of the samples which had an original count of more than 160,000 organisms per g. were less than 150,000 organisms per g. after 18 months of storage. This is shown in Table 12. This significant reduction in the number of organisms

TABLE 11

The number of organisms per gram in corn during 5 days' sampling after passing through various unit operations in plant GP

Source of sample	Number of samples	Plate count, incubated at 89.6° F. (32° C.)			Plate count, incubated at 131° F. (55° C.)		
		Highest count	Lowest count	Average count	Highest count	Lowest count	Average count
Before husker	8	10,000,000	1,000	1,384,000	16,000	0	2,000
After husker	8	23,400,000	8,000	4,324,000	2,000	0	200
After washer	8	40,000,000	850,000	5,830,000	4,000	0	900
After elevator, before cutter	8	30,400,000	950,000	6,175,000	4,000	0	1,000
After cutter and chute	8	32,600,000	3,300,000	11,422,000	6,000	0	200
After cleaner	7	58,000,000	5,300,000	10,711,000	4,000	0	800
After batch mixer	7	19,100,000	5,400,000	9,977,000	2,000	0	700
After blancher	7	1,000	1,000	1,000	2,000	0	200
After filler hopper	7	1,000	1,000	1,000	2,000	0	700
Final package	7	1,000	1,000	1,000	4,000	0	400

TABLE 12

Effect of storage on bacteria in 38 samples of corn with initial counts of greater than 160,000 organisms per gram

Period of storage	Temperature of storage	Samples under 150,000 organisms per g.
months		%
6	+10° F. (−12.2° C.)	69
12	+10° F. (−12.2° C.)	92
6	0° F. (−17.7° C.)	34
18	0° F. (−17.7° C.)	62
6	−10° F. (−23.3° C.)	35
18	−10° F. (−23.3° C.)	65

in frozen corn upon storage, even though the general count level may be higher than with peas and beans, may be a further indication that 150,000 organisms per g. may be eventually attainable as a maximum standard for corn.

At the lower temperature of freezing storage, as indicated in Table 13, the gas-producing types were widespread even in samples stored for 18 months. Again the greatest comparable reduction was at the +10° F. (−12.2° C.) storage.

TABLE 13

Percent of samples of corn showing gas-producing organisms[a] after freezing storage

Number of samples	Period of storage	Temperature of storage	Samples producing gas
	months		%
38	12	+10° F. (−12.2° C.)	24
38	18	0° F. (−17.7° C.)	75
45	18	−10° F. (−23.3° C.)	92

[a] Cultured in lactose broth medium.

Relation of count to quality of stored frozen corn. No relation could be found between ascorbic acid, thiamin, and riboflavin contents after storage and the original count of the corn. The only significant relation with respect to vitamin retention was greater loss of ascorbic acid at the higher storage temperatures.

Types of organisms in stored frozen corn. As shown in Table 6, 26 organisms were isolated from corn frozen at 0° F. (−17.7° C.) for 6 and 18 months and in contrast to the peas and beans the predominating types were *Micrococcus* sp. In contrast to peas and beans is the relatively small number of *Flavobacterium* types present, probably due to reduced contact of the corn with water which is the probable source of these types of organisms.

CONCLUSIONS

Little, if any, relation was noted between count on entering freezing storage and quality of peas, beans, or corn stored from 6 to 12 months at +10° F. (−12.2° C.), 0° F. (−17.7° C.), and −10° F. (−23.3° C.) when quality was defined by ascorbic acid, thiamin, carotene, and riboflavin retention and taste panel observations.

Greater reduction in count during storage was found at +10° F. (−12.2° C.), than at 0° F. (−17.7° C.) and −10° F. (−23.3° C.). There is evidence to indicate that although there is a relation between initial count and count after storage, the reduction is most pronounced in samples with higher initial counts. There are indications that a "base" cold-resistant flora is present in frozen peas, beans, and corn.

The mesophilic count on peas, beans, and born generally builds up prior to blanching. Proper sanitary procedures subsequent to blanching is an important factor in determining the number of organisms in the final package.

There did not appear to be a direct relation between visible sanitary condition of the equipment and the number of organisms present.

Micrococcus sp., *Flavobacterium* and *Achromobacterium* sp. and in some cases *Streptococcus* sp., were the predominating organisms in frozen peas, beans, and corn observed in the present studies.

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Microbiology of Frozen Orange Concentrate. I. Survival of Enteric Organisms in Frozen Orange Concentrate

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Stock cultures of Escherichia coli, Salmonella typhosa, and Shigella paradysenteriae inoculated into pasteurized orange concentrate and frozen were reduced so greatly in numbers within 24 hours a plate count on differential media could not be obtained. Within 48 hours the enteric pathogens could not be recovered by enrichment technique. No difference could be attributed to still or agitated freezing. The E. coli in fresh fecal material disappeared as rapidly under similar conditions indicating that the lack of survival was not due to the use of stock cultures. Streptococcus faecalis outlived all of the enteric pathogens and E. coli when added either from stock cultures or fresh fecal material. As a result it offers a possibility for use as an indicator of fecal pollution.

The acidity of orange concentrate and its storage at sub-zero temperatures has tended to obscure the fact that neither low pH nor low temperature can insure the destruction of pathogenic organisms. The possibility exists that pathogens, particularly those of fecal origin, introduced into citrus concentrates through the medium of flies, handlers, polluted wash water, etc., might remain viable after freezing.

A series of experiments was designed to study the survival of enteric organisms in frozen concentrate, the effect of still freezing versus agitated freezing and a comparison of the longevity of *E. coli* and *Str. faecalis* as possible indicators of fecal pollution. While there is little literature specifically dealing with the microbiology of frozen citrus concentrates some work has been published on the survival of micro-organisms in single-strength orange juice. Beard and Cleary *(1)* found that strains of *S. typhosa* survived 7 days when inoculated into orange juice (pH 3.5) and stored at —4° C., whereas, the same strains introduced into broth at the same pH and stored at 37.5° C. were destroyed within 24 hours. They concluded that the bactericidal effect of the hydrogen ion was appreciably inhibited at low temperatures.

Schrader and Johnson *(6)* studied the behavior of *E. coli* in frozen orange juice held at various temperatures and observed that the death rate decreased as the temperature was lowered. They concluded, however, that *E. coli* would fail to survive longer than 2 weeks in frozen orange juice.

Wolford *(7)* examined 79 samples of commercially prepared frozen orange juice stored at —23.3° C. for 43 weeks. During the entire 43-week storage period he found viable coliforms, many of which resembled *E. coli*.

Faville, Hill, and Parish *(3)* inoculated pure cultures of *E. coli*, *Leuconostoc mesenteroides*, and an *Achromobacter* species into orange concentrate. These organisms were greatly diminished in number at 30° C. and 40° C. within 7 hours, whereas only *E. coli* showed an appreciable reduction in numbers when the concentrate was stored at 3° C. for the same time.

The results of investigations on the survival of micro-organisms in single strength orange juice are not necessarily applicable to orange concentrate since factors which might effect microbial viability are introduced by concentration of the juice. The approximate 300% increase in solid constituents which results from concentrating orange juice may afford protection to micro-organisms which failed to survive in single strength juice. McFarlane *(5)* tested the effect of increased sucrose concentration on the survival of organisms in orange juice stored at —17.8° C. and observed that greater destruction of cells occurred in unsweetened juice than in juice containing added sugar. On the other hand, in orange concentrate there is an increase in the concentration of organic acids, mostly citric, and this might be expected to increase the lethal action of concentrate as compared to single-strength juice. The increased osmotic presure may also influence microbial viability.

When orange juice or concentrate is frozen there is a tendency for the solids to migrate to the central core of the container. In slow freezing this effect is greatly exaggerated. McFarlane *(4)* demonstrated that microbial forms retained their viability in those sections of a frozen mass which possessed a high concentration of favorable substances such as sucrose.

EXPERIMENTAL PROCEDURE AND RESULTS

The orange concentrates used were commercial products.[b] Since quantitative results were desired, the juice was pasteurized to destroy yeasts and molds which would interfere with plate counts. Cans of concentrate were thawed in cold running water then placed in a hot water bath until the center of the containers reached 60° C. after which they were held at this temperature for 30 minutes.

The organisms used in this study were, except where otherwise indicated, stock strains of *Escherichia coli*, *Salmonella typhosa*, *Shigella paradysenteriae*, and *Streptococcus faecalis*. To test for survival in frozen orange concentrate 9-ml. portions of pasteurized juice were pipetted into sterile culture tubes to which were added 1-ml. amounts of 24-hour broth cultures of the test organisms in appropriate dilution. The tubes were shaken then placed in a freezer at —17° C. At 24-hour intervals the required number of tubes were thawed in cold water after which the juice was neutralized with sterile 1 *N*. NaOH and plated out to determine the number of viable cells. Tubes of juice once thawed were not used again. Spreading colonies of unidentified Gram positive spore forming bacteria which had apparently survived pasteurization necessitated the use of media which inhibited the growth of these organisms. E. M. B. agar (Difco) was used in the recovery of *E. coli;* S. S. agar (Difco) for *S. typhosa* and *Sh. paradysenteriae;* and S. F. medium (Difco), into which agar had been incorporated, for *Str.*

[a] Present address: Rexall Drug Co., Los Angeles, California.

[b] Courtesy of Bireley Division, General Foods Corp.; California Consumers Corp.; and Exchange Orange Growers Corp.

faecalis. Plates were incubated a minimum of 24 hours at 37° C. before counts were made. A further attempt to recover the enteric pathogens was made by inoculating portions of the thawed concentrate into tubes of Difco tetrathionate broth. These were incubated for 18 hours at 37° C. at which time streaks were made from these to S. S. agar plates which were incubated at 37° C. for 24 hours. If typical colonies appeared and were found to be Gram negative, non-sporulating rods, it was assumed that some of the enteric organisms were still viable.

The bactericidal effect of frozen orange concentrate on *E. coli*, *S. typhosa*, and *Sh. paradysenteriae* is apparent from the inability to recover any of these organisms after 24 hours of storage in concentrate at —17° C. (Table 1). Failure to obtain growth

TABLE 1

Survival of enteric organisms in orange concentrate stored at —17° C.

Storage time in hours	Plate count per ml.					
	Fecal suspensions in		Pure cultures			
	EMB Agar	SF Agar	E. coli	S. typhosa	Sh. paradys.	Str. faecalis
0	39,000	11,000	3,900	4,200	3,600	3,600
24	0	31	0	0[c]	0[c]	445
48	0	16	0	0[c]	0[c]	233
72	0	18	0	0[c]	0[c]	220

[c] Negative after enrichment in tetrathionate broth.

of *S. typhosa* and *Sh. paradysenteriae* after inoculation of the thawed juice into enrichment broth gave further evidence of the lethal action of orange concentrate on these organisms.

A study was made to determine whether the increasing concentration of solids which migrate to the center of the can in freezing exert a protective effect on microbial activity. Test organisms were inoculated directly into a series of cans of pasteurized concentrate, the holes were then sealed with hot paraffin and the cans were shaken vigorously and immediately placed in a freezer. One series of cans was agitated during the freezing process while a second set was allowed to still-freeze. At 24-hour intervals individual cans were thawed and aliquots of the concentrate were plated on appropriate media.

Still-freezing cans of orange concentrate into which the test organisms had been introduced had no apparent protective effect on microbial viability as can be seen from the data in Table 2.

TABLE 2

Effect of agitation during freezing on the survival of enteric organisms in frozen orange concentrate stored at —17° C.

Organism	Plate count per ml. Storage time in hours			
	0	24	48	72
E. coli				
Agitated-frozen	575,000	0	0	0
Still-frozen	575,000	0	0	0
S. typhosa				
Agitated-frozen	465,000	0[d]	0[e]	0[e]
Still-frozen	465,000	0[d]	0[e]	0[e]
Sh. paradysenteriae				
Agitated-frozen	410,000	0[d]	0[e]	0[e]
Still-frozen	410,000	0[d]	0[e]	0[e]
Str. faecalis				
Agitated-frozen	515,000	13,500	10,000	9,100
Still-frozen	515,000	12,000	15,000	8,200

[d] Positive after enrichment in tetrathionate broth.
[e] Negative after enrichment in tetrathionate broth.

Attempts to recover *E. coli* at 24 hours from either still-frozen or agitated frozen cans were negative by plating techniques, however, no attempt was made to obtain these organisms by enrichment cultures. The fact that *S. typhosa* and *Sh. paradysenteriae* could be recovered after 24 hours' storage in frozen concentrate by inoculating the juice into tetrathionate broth can be attributed to the heavy initial inocula since viable organisms were recovered in both the still-frozen cans and those which had been agitated during the freezing process. However, in spite of the large inocula used, no organisms could be recovered from any of the cans after 48 hours' freezing storage.

The ability of *Str. faecalis* to survive in frozen concentrate is evident from the data in Tables 1 and 2. This is in marked contrast to the rapid destruction of *E. coli* which could not be recovered after 24 hours' storage even when inoculated at the rate of 0.5 million cells per ml. of concentrate. The enterococci exhibited a large initial drop in numbers within 24 hours but the subsequent decline was gradual and viable cells could be recovered after 3 weeks of storage at which time the experiment was terminated.

Dilute suspensions of fecal material were inoculated directly into pasteurized concentrate to determine whether the *E. coli* strains in feces would be more viable than the stock strains. The concentrate received 39,000 coliforms and 11,000 fecal streptococci per ml. as determined by plating on E. M. B. and S. F. media. The concentrate was frozen immediately and samples were thawed, neutralized, and examined at 24-hour intervals for 3 days using the same media. No coliforms could be detected at any time; however, the results for fecal streptococci were positive at each sampling period.

DISCUSSION AND CONCLUSIONS

The presence of coliform organisms in water is assumed to indicate pollution of a water supply. The same conclusion may not be drawn in regard to orange concentrate since most of the coliforms are of non-fecal origin and the fecal strains tested do not survive in concentrate under the conditions of this test. The inability to recover coliform organisms from orange concentrate inoculated with fecal material would indicate that coliforms reported to have been isolated from orange juice after long storage periods were probably not of fecal origin.

The fact that the pathogens tested failed to survive long periods of storage in concentrate does not eliminate the need for methods to determine fecal contamination of citrus products. There is always the possibility that other enteric pathogens might be less sensitive to freezing in orange concentrate; also, there are aesthetic objections to the presence of fecal matter in food. The inability of enteric pathogens such as *S. typhosa* and *Sh. paradysenteriae* to survive freezing in orange concentrate would seem to indicate that there is little danger of infection by these organisms through frozen concentrate. This is especially true since massive contamination of commercial orange juice would be relatively rare.

Organisms belonging to the enterococci are claimed to be of intestinal origin and work has been done indicating the superiority of that group of organisms to coliforms as indicators of fecal pollution in frozen food. For example, Burton *(2)* examined samples of commercially frozen vegetables and cantaloupes for fecal contamination and concluded that fecal streptococci are superior in their ability to survive freezing storage. The results of these experiments are in agreement that the survival of strains of *Str. faecalis* greatly exceeds that of *E. coli* or the enteric pathogens. Whether this fact alone should govern the choice of this organism as an indicator of fecal pollution is a matter of debate. Work still in progress at this Department indicates that this is an extremely complex problem and conclusions should not be drawn without sufficient experimental data.

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Book Review

Advances in Enzymology. Volume XII. Edited by F. F. Nord, 570 pages, with Cumulative Author and Subject Index for Volumes I-XII. Interscience Publishers, Inc., New York, N. Y., 1951. Price $9.75.

The current book equals the high standards set by the earlier volumes of this series. It contains the following 9 chapters:

Oxidoreduction in Chloroplasts, by Robert Hill.
Mechanisms of Fixation of Carbon Dioxide by Heterotrophs and Autotrophs, by Merton F. Utter and Harland G. Wood.
Enzyme-Substrate Compounds, by Britton Chance.
The Specificity of Certain Peptidases, by Emil L. Smith.
The Enzymic Hydrolysis and Synthesis of Acetylcholine, by David Nachmansohn and Irwin B. Wilson.
The Present Status of Starch Chemistry, by Kurt H. Meyer and G. C. Gibbons.
Enzymes of Starch Degradation and Synthesis, by Peter Bernfeld.
Biological Methylation, by Frederick Challenger.
Reaction of Borate with Substances of Biological Interest, by Charles A. Zittle.

The chapters are extremely well documented and will serve adequately to orient the reader in the several fields. The book will appeal especially to biochemists who are engaged in research in the areas covered; it should also be invaluable for the teacher. The information is brought very much up to date. In most chapters, references include publications as late as 1951. The chapters on the chemistry of starch and on the enzymes involved in the degradation and synthesis of starch will be of interest to many food technologists in the baking and milling industries.

Harry J. Deuel, Jr.

Corrosion Guide. By E. Rabald. 629 pages. Elsevier Publishing Company, New York, Amsterdam, London, Brussels. 1951. Price $12.50.

This book consists of three chapters of 46 pages covering general considerations, principles of corrosion and measurement of corrosion resistance and approximately 570 pages of tables listing the corrosive action of 250 substances, mostly chemicals, on approximately forty different possible construction materials including aluminum, aluminum-silicon alloys, copper, brass, bronze, aluminum bronze, copper-silicon, iron, steel, stainless steels, silicon steel, lead, nickel, nickel alloys, silver, tin, ceramic materials, plastics, glass lined steel, rubber, porcelain, concrete, wood and some miscellaneous materials. There are also 10 pages of bibliography. It is written primarily for chemical manufacturers, most of the substances listed in the tables being chemicals. Among the materials listed which may be of interest to the food technologist are citric acid, dextrose, fats, fruit juices, gelatin, molasses and sugar solutions.

Resistance of the materials to corrosion is indicated by one of four designations, namely, "practically resistant," "fairly resistant," "not particularly resistant but used" and "unusable" each of which is defined in terms of g./m.^{2} of material dissolved per day. In many instances data are given on corrosion at elevated temperatures.

The book is well printed and the tabular matter is given in a form very convenient for use. Although the corrosion data on food materials are mostly limited to those items listed above, the book will be of use to food technologists dealing with these products or who encounter in their operations solutions of almost any of the common chemicals.

A. E. Stevenson.

The Master Dictionary of Food and Cookery and Menu Translator. By Henry Smith. 263 pages. Philosophical Library Publishers, Inc., New York, N. Y., 1950. Price $2.50.

This book was made and printed in Great Britain. The quality of the physical format shows the austerity of British conditions. Since the book is a dictionary of food and cookery terms there is no table of contents and since the terms defined are arranged in alphabetical order, no index is needed.

The book was written for people of Great Britain and does not serve as well therefore for residents of the U. S. Some of the terms are not used in this country to any extent, if at all. Gingerbread is defined as "one of the Christmas festival breads and is connected with English fairs to the present time."

The book is not a suitable one to put in students' hands because there are too many mistakes. For example, levulose is defined as corn syrup and honey as well as fruit sugar; angel cake is referred to as a confection. Probably the term confection has a different meaning in Britain than it does in this country. Early failures in angel cakes are ascribed to the fact that "a special soft flour was required to insure lightness and soft eating qualities." The fact is that a good angel cake can be made with bread flour.

Crepe suzettes are defined as pancakes. Giblets are referred to as poultry or game bird offal. Some of what we may term as mistakes are no doubt due to differences in meaning of the same word in the U. S. and in

Britain. The fact that the book was written by a chef and consultant on food and cookery and not by a food technologist is obvious.

While half of one column is given to gingerbread no mention is made of the fact that it is a quickbread. Often the history of the food is given with the omission of a good definition of the term, in spite of the fact that the book is called a dictionary.

The best use of the book would be in the hands of an experienced food person who already has enough background to sift the good from the poor.

The term *Menu Translator* in the title is meaningful and describes aptly a large part of the book. Many of the terms included in the book are French; some are German, Italian, Latin and Russian.

FAITH FENTON.

Notes and Letters

A Comparison of Two Methods for the Bacteriological Examination of Shell Eggs

As has previously (1950) been pointed out *(2)*, it is difficult, if not impossible, to make a completely satisfactory bacteriological examination of shell eggs. The variations resulting from differences in technic are especially important in determining the presence or absence of bacteria. The work herein presented compares a plating method as closely as possible to the method used in this laboratory and described in an earlier publication *(2)*.

PROCEDURE AND METHODS

The eggs used in this series of experiments were taken at random from current receipts held in the egg cooler. They were from one day to several days old. Sometimes the fresher eggs were held for 2 or 3 days at room temperature before being examined to improve the chance of getting contaminated eggs. Samples of 24 eggs each were used for comparison. The eggs were dipped in saturated $HgCl_2$ and carefully flamed. The contents were then emptied into sterile, rubber-stoppered, wide-mouthed jars, each containing a small quantity of glass beads. After the jars were thoroughly shaken, approximately 11.0 cc. of the egg mixture were pipetted into a 99 cc. water blank, using an 11 cc. pipette with the tip cut off. (The method suggested by the American Public Health Association *(1)* calls for weighing out 11 g. of egg for plate counts, but it was felt that such a procedure would be more laborious and exacting than necessary for a qualitative bacteriological determination). From the 1-10 dilution, 1 cc. and 0.1 cc. portions were plated out with tryptone glucose extract agar. The plates were incubated at room temperature for 3 days. To the remaining portion of egg in each jar, 25 cc. of double strength thioglycollate broth were added, and the mixture remained at room temperature for 3 days. At the end of that time, the plates were counted and the jars were examined; the obviously positive jars were noted. From the remaining jars, transfers were made into thioglycollate broth with a 4 mm. loop. These tubes were incubated at room temperature for 3 days, and the presence or absence of growth recorded.

Over a period of several months, 459 eggs were examined. As shown in Figure 1, 256 or 55.7% were positive by the thioglycollate culture method while only 169 or 36.8% were positive by the agar plate method. Only 124 or 27% were identically positive by both methods. The total positive by either method was 301 or 65.5%. Of the eggs reported as positive by the plate method, 30% were negative by the thioglycollate culture method, while 51% of the thioglycollate positive eggs gave no colonies on agar plates.

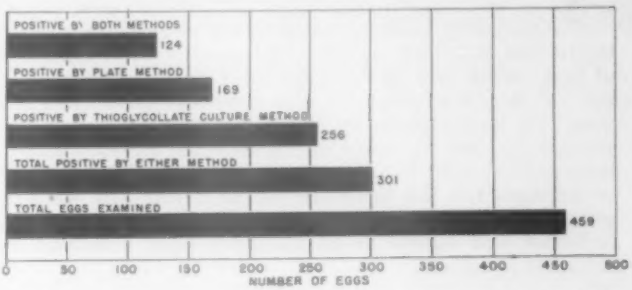

Figure 1. A comparison of two methods for the bacteriological examination of shell eggs.

DISCUSSION

The results obtained in these experiments seem to show a distinct advantage in the use of the whole egg as an inoculum for sterility tests. There is, of course, the possibility of getting false positive results from stray organisms in the egg-thioglycollate broth mixture. On the other hand, there is also the chance of contamination of agar plates. It should also be remembered that the removal of about one-fifth of the egg for the plate count lessened the chance of recovering bacteria by adding thioglycollate broth to the remaining portion. On the whole, it appears that the use of the whole egg as inoculum provides a simple yet accurate measure of the sterility of fresh eggs.

SUMMARY

A comparison between plating aliquots of whole egg and inoculating the entire egg into thioglycollate broth gave results in favor of the latter method.

JACK WOLK
Bureau of Animal Industry,
U. S. Department of Agriculture,
Beltsville, Md.

October 12, 1951.

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The Color in Cranberries

Grove and Robinson *(1)* isolated a pigment from Newfoundland and Cape Cod cranberries and identified it as the anthocyanin 3-β-glucosidyl peonidin. This has been generally accepted as representing the entire color in cranberries.

When strained cranberry sauce is first canned, the liquid that exudes as the result of syneresis is brilliantly red. After some months of storage this liquid is almost devoid of color, the time required for the loss of color being decreased by increasing storage temperatures. In the meantime the sauce loses its brilliance, in trade parlance darkens, but is more representative of the sauce as it usually reaches the consumer. If such a sauce is dispersed in water and filtered, a filtrate almost devoid of color results. If, however, the sauce is dispersed in an equal volume of acetone it yields a brilliantly red filtrate.

The cranberry juice of commerce is obtained by pressing berries that have been in frozen storage without the application of heat. While this juice is deep red, the press-cake seems no less red than the berries. If this press-cake is submerged in water, the berries plump up after a few hours to almost their original size, and the liquid then pressed out has only about a tenth as much color as the original juice. If this is repeated five or six times, the final liquid pressed out is very pale, while the press-cake seems to lose none of its color. If now the press-cake is treated with acetone or alcohol and pressed, a brilliant red extract is obtained.

It thus appears that the cranberry has both a water-soluble and a water-insoluble, acetone-soluble red pigment. It is well recognized that the anthocyanins are hydrolyzed in such acid fruits as the cranberry and the loganberry, and the anthocyanidin is precipitated as brown sediments. This may account for the so-called darkening of commercially canned cranberry sauce. The red color of the sauce is then maintained by the more stable water-insoluble pigment.

Some preliminary data suggest, however, that the basis of this color may be the same as in the water-soluble form. This is indicated by some chemical reactions as well as paper chromatography. The pigment must then be in a different physical form, possibly in a loose combination. The rather abundant characteristic wax in cranberries makes this seem a plausible explanation, particularly since in the sound cranberry all the color is in a thin layer just under the skin, which is coated with the wax.

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Edward F. Kohman.

February 21, 1952.

Maceration vs. Comminution

To the Editor of *Food Technology:*

The verb "macerate" [see *Food Technology*, **6**, 68 (1952)] should be used correctly or we must change the dictionaries. It does *not* mean mash, pulp, sieve, screen, comminute, triturate or otherwise divide mechanically into small particles. "Concise Chemical and Technical Dictionary," 1947, defines maceration as "the process of steeping or swelling a solid in a liquid, so as to cause softening, often with separation of the parts; extraction of drugs; preparation of iron ore for smelting by exposing it to the weather long enough to remove all sulfur." Hackh's "A Chemical Dictionary" defines macerate "The steeping or softening and breaking up of a solid by soaking in water."

Webster indicates that the steeping results in separation of fibres. The first examples that come to mind are retted flax and over-cooked pot roast.

To bring the matter entirely up to date, Dr. Frank Colby was consulted shortly before his recent death. His syndicated column, now conducted by his wife and son, appears in many newspapers. He was a strong advocate of requiring the dictionaries to reflect current usage. To the question, "Does maceration mean or imply trituration or mashing, chopping or other means of disintegration?" he replied, underlining trituration, mashing and chopping, "No. It means to soften by steeping in a liquid, with or without heat; to wear away or separate the parts of, by steeping; to soften or wear away by digestive or other physiological process."

The A.O.A.C. uses the term "comminuted" to describe tomato products that have been forced through a screen, and since the word is entirely adequate why confuse readers by introducing a term already in use with different meaning!

S. H. Champlin
c/o The Heekin Can Company
Norwood, Ohio

February 7, 1952.

None of the reviewers of this paper, representing people well experienced in tomato technology, objected to our use of the word "maceration" which has been employed by plant physiologists for decades in a meaning not found in the dictionaries. It indicates the destruction of cell structure and continued agitation of the resulting mixture which, by this time, is composed of cellular debris of all sorts suspended in a liquid (cell sap). During this proces many other reactions resulting in chemical and physical changes occur. Comminution, to us, indicates the reduction in particle size but this is only one of the many reactions taking place when the original organization of tomato tissue is destroyed. Neither word is entirely suitable and adequate, and after considering several terms, including "comminution," our choice was "maceration." It is hoped that eventually somebody will suggest or invent a better word than either "maceration" or "comminution" for describing what occurs in this process.

Lillian B. Kimball and Z. I. Kertesz.

February 27, 1952.

Rendering Lard at Low Temperatures[a]

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(Manuscript received January 16, 1952)

Leaf and skin-free cutting fats were rendered at low temperatures by comminution, heating, agitation, and filtration. Information was obtained and is presented on: 1, significant factors in carrying out various unit operations; 2, optimum rendering conditions from the standpoint of yield and quality of product; and 3, the color, odor, flavor, and stability of the lard produced.

It is widely recognized that present lard-rendering methods generally employ heat to an extent that is detrimental to the color, odor, and flavor of the product. To improve the quality of lard and increase its economic value, renderers have been seeking, and in some cases introducing improved rendering and processing methods. Part of the industry believes that the solution of the problem lies in processing lard as a raw material in the production of "tailor-made" shortenings. However, under present conditions, it has not been practical for large packers to process extensively the bulk of the lard, nor are practical means available for effectively processing it on farms, at locker plants and by small packers. A large part of the industry, consequently, has shown considerable interest in the possible development of rendering processes capable of directly producing lard of more acceptable color, odor, and flavor.

A number of new rendering processes that may subsequently prove of value to the industry have been developed *(1, 2, 3, 4, 6, 7, 9)*. By employing such operations as mechanical comminution, rapid steam cooking *(1)*, solvent extraction *(9)*, digestion with alkali *(2, 3)* or enzymes *(6)*, heating under varying pressures *(4)*, and rapid "shock" heating *(7)*, the new processes require fat and fat tissue to be heated only to a limited extent. As previously indicated in a review of these methods *(8)*, it is unfortunate that relatively little information is available on which evaluation of the processes can be based. Of equal or perhaps greater importance is the lack of information on the factors involved in efficiently utilizing various types of operations that would provide an adequate basis for improvement and development.

The present investigation is primarily concerned with the development of improved rendering processes having the widest possible application in the field of fat rendering. Attention was initially directed to employing comminution, heating, agitation, and filtration, in rendering skin-free fat at low temperatures by a modified open kettle process. Pilot-plant equipment was used. The specific objectives were to obtain information on: 1, significant factors in carrying out various unit operations; 2, optimum rendering conditions from the standpoint of yield and quality of product; and 3, the color, odor, flavor, and stability of the lard produced.

[a] This work was done with funds made available by the Research and Marketing Act of 1946.

METHODS, MATERIAL, AND EQUIPMENT

The leaf and skin-free cutting fats for rendering were obtained from hogs of approximately 225 pounds live weight. The fatty tissue was stored at 37° F. (2.8° C.) and rendered 1 to 20 days after slaughter. The free fatty acid content of the lipids in the stored tissue increased at a rate of 0.08% per day. A meat grinder (800 pounds per hour capacity) equipped with size 32 grinder plates (3.94-inch diameter) and driven by a 2.0 h.p. electric motor was used in grinding. Rendering was accomplished in a stainless steel kettle (20-gallon capacity) that had a steam-jacket around the bottom and lower half. Mechanical agitation was supplied by a portable agitator driven by an electric motor (0.25 h.p.) at 1725 r.p.m. The agitator was equipped with two 3-blade propellers; the elliptically-shaped blades extended approximately 2 inches from the propeller shaft. Filtration was accomplished through single layers of cloth obtained from feed bags, the fabric corresponding to unbleached sheeting, warp, 64 per inch; filling yarn, 60 per inch; weight, 4.5 ounces per square yard. With a few exceptions, which are noted, 70- to 72-pounds batches were ground through a plate with 0.125-inch openings, after which they were rendered. The rendered batches were filtered by pouring them on approximately 2 square feet of the above-described cloth, which separated the residues of connective tissue and incompletely rendered tissue from the lard.

The moisture content of samples of lard was determined by Method Ca 2b-38 and the Lovibond color of samples by Method Cc 13b-45 of the Official and Tentative Methods of the American Oil Chemists' Society *(5)*. Stabilities were determined by the active oxygen method (A.O.M.) at 208° F. (97.7° C.). The lipid content of samples of the residues was calculated from data obtained by extracting the lipids from samples of oven-dried residue as follows: The oven- dried residue was washed with chloroform (30 ml.) and twice extracted with the solvent in a Waring Blendor, which disintegrated tissue and aided extraction. The 3 chloroform extracts (total volume approximately 175 ml.) were combined and filtered; the solvent was evaporated from the lipids on a steam bath; the lipids were then heated at 230° F. (110° C.) under vacuum for 3 hours, cooled and weighed. Calculations of the lipid content of samples of original residue were based on the weights of extracted lipids and the weights of original samples. Yields based on the lipid content of the raw fats rendered were calculated by the formula:

$$\text{Yield based on the lipid content of raw fat, \%} = \frac{\text{Wt. lard rendered, pounds}}{(\text{Wt. residue, pounds}) \times (\text{lipid content of residue, \%}) + (\text{Wt. lard rendered, pounds})} \times 100$$

The lipid content of the raw fats used in the experiments was calculated by the following formula:

$$\text{Lipid content of raw fat, \%} = \frac{(\text{Wt. residue, pounds}) \times (\text{lipid content of residue, \%}) + (\text{Wt. lard rendered, pounds})}{\text{Wt. raw fat rendered, pounds}} \times 100$$

Yields based on the weight of raw fat rendered were calculated by the following formula:

$$\text{Yields based on weight of raw fat, \%} = \frac{(\text{Wt. of lard rendered, pounds})}{(\text{Wt. raw fat rendered, pounds})} \times 100$$

PROCEDURE AND RESULTS

Yields in Relation to Rendering Operations

Series 1. Series 1 consisted of experiments to determine the effect on yields of grinding fat tissue through different plates. A 120-pound lot of backfat was separated into 4 30-pound batches,

each of which was ground through a different plate; the plates had 0.125-, 0.25-, 0.5-, and 1.0-inch openings (diameter). Each batch was placed in the kettle, heated to 167° F. (75° C.) and held at this temperature for 10 minutes. Steam at 40 pounds p.s.i.g. was used in initially heating the batch; very little steam was necessary to maintain the temperature. Mild agitation (manual stirring) was maintained to prevent scorching. After 10 minutes had elapsed, the batch was discharged on a filter, which separated it into tissue and lard fractions.

The results of the experiments including information with respect to the lipid content of the raw materials and yields are given in Table 1. The results show that wide variations in the dimensions of the openings through which the tissue was ground affected yields to a relatively small, but important, extent.

TABLE 1

Effect of grinding with different plates on yields of lard

Diameter of openings in grinder plates	Lipid content of fat rendered	Yield, based on weight of raw fat	Yield based on lipid content of raw fat
inches	%	%	%
1.0	86.0	70.0	81.4
0.5	85.9	73.8	85.6
0.25	89.0	76.3	85.6
0.125	88.7	79.1	89.2

Series 2. Series 2 consisted of experiments dealing with the effect on yields of grinding fats at different temperatures. Batches from different lots (A, B, C, and D) of cutting fats and from one lot (E) of leaf fat, after initial storage at 37° F. (2.8° C.), were stored for at least 24 hours at 0° F. (—17.8° C.) to 37° F. (2.8° C.), with or without subsequent warming at room temperature. The fat comprising each of the various batches was rendered as follows: The fat was ground through a 0.125-inch plate and the temperature of the ground fat was determined. The kettle was heated with steam at 50 pounds p.s.i.g., the agitator was started and the ground fat was then added to the kettle in 4 to 5 pound portions. When the batch had been heated to 194° F. (90° C.) the steam valve was closed and heat from the walls of the kettle was allowed to heat the batch to 203° F. (95° C.). The charging operation required approximately 3 minutes; heating, measured from the time charging was begun, required approximately 8 minutes. The batch was immediately discharged into a galvanized iron drum (10-gallon capacity) where it was allowed to stand until it was poured on the filter 20 minutes later. Data and information on the lipid content, storage and post-grinding temperatures of the various batches of fat and the moisture content and yields of the lard as shown in Table 2.

The results indicate that yields were affected to a marked extent by variation of the temperature of the fat tissue when fed into the grinder. Cooling or freezing the tissue prior to grinding increased yields, better yields being obtained on cooling to 0° F. than to 26° F.

TABLE 2

Effect of varying the temperature at which fat was stored prior to grinding on yields of lard

Lot designation	Batch number	Temperature of storage of fatty tissue	Temperature of freshly ground fat	Lipid content of fatty tissue	Moisture content of lard	Yield[b]
		° F.	° F.	%	%	%
A	1	0	28	90.0	0.14	96.6
	2	37	54	89.9	0.09	83.2
	3	37[c]	61	89.2	0.17	76.6
B	4	26	46	89.0	0.24	90.0
	5	26[c]	57	89.6	0.09	81.7
C	6	37	50	87.7	0.17	87.9
	7	26	46	88.7	0.19	91.2
D	8	0[d]	57	86.6	0.16	79.2
	9	0	26	87.1	0.13	95.7
E	10	0	28	91.2	0.16	98.4
	11	37	52	91.2	0.13	98.0

[b] Yield based on the lipid content of raw fat.

[c] Fat was allowed to warm by standing at room temperature prior to grinding.

[d] Fat was stored for 2 days at 0° F. followed by storage for 2 days at 37° F.

or higher temperatures, except that cooling to 0° F. (—17.8° C.) instead of 37° F. (2.8° C.) did not affect the yield in rendering leaf fat. Relatively poor yields were obtained in rendering fat tisue that had been frozen and allowed to warm, which fact suggests that such rupture of cells as may have occurred during freezing did not significantly affect yields. Rather, it appears logical to associate the high yields obtained on rendering chilled or frozen fats with the relative brittleness and rigid cell structures of frozen or chilled tissue which, consequently, becomes more thoroughly sheared during grinding.

Series 3. Series 3 consisted of experiments to determine the effect on yields of rendering at different temperatures and with different rates of heating. Here, 4 lots (F, G, H, and I) of cutting fats were rendered. Lots F and G were separated into 3 batches each, which were rendered at different maximum temperatures. Otherwise, all batches were handled alike. Lots H and I were separated into 2 batches, each of which was rendered under comparable conditions except that 1 batch from each lot was heated with steam at low pressure and the other with steam at relatively high pressure. The steam pressures ranged from 0 to 50 pounds p.s.i.g. The rendering procedure corresponded to that described above (Series 2), except that the 20-minute period of standing was omitted. The results of the experiments, including data on yields and details of the rendering operations, are given in Table 3.

The results show that, under the conditions studied, neither variation of the maximum temperature obtained

TABLE 3

Relations between rendering temperatures, rates of heating, and yields of lard

Lot designation	Batch number	Temperature of ground fat	Rendering temperature	Steam pressure	Heating period	Lipid content of raw fat	Moisture content of lard	Yield*
		° F.	° F.	*p.s.i.g.*	*minutes*	%	%	%
F	12	32	169	50	6	88.2	0.36	95.2
	13	34	183	50	7	89.0	0.37	94.9
	14	32	203	50	8	88.0	0.39	96.6
G	15	30	145	45	6	89.8	0.23	94.7
	16	32	195	45	8	91.2	0.23	97.3
	17	32	216	45	9	89.9	0.35	96.9
H	18	34	201	50	8	90.5	0.22	96.0
	19	34	201	0-5	36	90.8	0.22	95.8
I	20	50	185	40	7	90.0	0.24	84.1
	21	50	185	10	15	90.1	0.27	85.3

* Yield based on lipid content of raw fat.

during rendering nor variation of the rate at which the batches were heated appreciably affected yields. The pink color and gel-like consistency of the cellular tissue changed as the temperature was raised higher than 131° to 140° F. (55° to 60° C.) and it became relatively firm and acquired a light amber color. The amount of moisture evaporated by the heating was not determined; however, during the entire rendering operations the average loss of weight per batch was 1.4%. Consequently, the moisture that evaporated was not more than, and probably much less than, 20% (1.4/7.4 × 100) of the original moisture in the fat tissue. The temperature of the kettle walls (0 to 50 pounds p.s.i.g. corresponds to 212° to 297.5° F. or 100° to 147.5° C.) in the present study ranged from substantially lower than, to almost as high as, 300° F. (149° C.), which temperature has been reported to be capable of producing a "cracking" effect on fat cells *(7)*.

Series 4. Series 4 consisted of experiments to determine the relation between agitation and yields. Each of 2 lots (J and K) of cutting fats was separated into 2 batches. One batch from each lot was manually agitated and the other batch was mechanically agitated during rendering operations, which were otherwise comparable. The rendering procedure was that described above (Series 2), except that the 20-minute period of standing was omitted. Data and information with respect to yields, the moisture content of samples of the rendered lard, and details of the rendering operations are shown in Table 4.

It was found that somewhat larger yields were obtained from mechanically agitated batches. Manual stirring, although sufficiently vigorous to prevent scorching the fat, nevertheless failed to adequately disintegrate lumps and aggregates of tissue.

Series 5: In this series 5 batches from different lots of cutting fats were rendered at 203° F. (95° C.) in the kettle heated with steam at 40-50 pounds p.s.i.g. The rendering procedure was that described above (Series 2). The yields obtained, based on the lipid content of the raw materials, averaged 88.8%. Centrifuging residual tissue in a basket-type centrifuge increased the yields to an average of 92.4%. Disintegration of the residues in a Waring Blendor increased the average yield to 96.0%. Then 5 batches of leaf fat were rendered by the above-indicated procedure, with an average yield of 96.2%. Centrifugation of the residue obtained in rendering leaf fat increased the total yield to an average of 98.8%. Subjecting the residue to further disintegration in the Waring Blendor, however, did not produce additional lard.

Then 8 batches of cutting fats that had been stored for 1 to 2 days at 0° F. (—17.8° C.) after initial storage at 37° F. (2.8° C.) were rendered at 203° F. (95° C.) with steam at 40-50 p.s.i.g. The rendering process described in Series 2 was followed. The yields ranged from 95.2 to 96.8%, the average yield being 96.0%.

Each of 4 other lots of cutting fats was divided into 2 batches 1 of which was rendered as described in Series 2 and the other by the open kettle method at 240° F. (115.5° C.). The cracklings produced by the latter method were not pressed. Data obtained are given in Table 5, which shows that the low temperature method yielded 2.9 to 5.9% more lard under the conditions investigated.

TABLE 5

Comparison of yields obtained by the low temperature and open kettle[g] methods of rendering

Lot designation	Batch number	Rendering method	Lipid content raw material	Moisture content of lard	Yield[h]
			%	%	%
L	26	L.T.[i]	90.7	0.34	96.6
	27	O.K.[j]	89.0	0.17	93.7
M	28	L.T.	87.0	0.32	95.0
	29	O.K.	86.2	0.29	89.1
N	30	L.T.	89.7	0.23	96.2
	31	O.K.	89.8	0.11	92.0
O	32	L.T.	89.9	0.21	97.1
	33	O.K.	89.1	0.09	93.0

[g] Cracklings obtained by the open kettle method were not pressed.
[h] Yield based on lipid content of raw fat.
[i] Low temperature method.
[j] Open kettle method.

Methods of Reducing Moisture in Lard

The moisture content of samples of lard rendered at low temperatures was as high as 0.4%. Approximately 50 samples containing 0.2% or more of moisture were stored for a month or longer; the detection of souring or mold formation in 5 of these directed attention to the need of reducing the moisture content of the lard to avoid occasional spoilage. Attempts to remove excess moisture from the lard by drying it at 158° to 176° F. (70° to 80° C.) affected its odor and flavor. It was found that the lard could be dried at the above temperatures without significantly affecting its odor and flavor if it was first water-washed by adding one half volume of water at 158° F. (70° C.), vigorously agitating the mixture and settling out the wash water. The batches were given 2 such washes.

A fairly satisfactory reduction of the moisture content of lard was also obtained by allowing the tissue and lard to stand, with slow stirring, before separating them by filtration. The effects obtained by such a procedure are shown in the results of the following experiment.

A batch of ground cutting and leaf fats was rendered at 201° F. (94° C.). A 1-pound sample of the lard was withdrawn and filtered. Its moisture content was 0.34%. One half of the batch was discharged into a

TABLE 4

Relation between degree of agitation of fat and yield of lard

Lot designation	Batch number and type of agitation	Temperature of ground fat	Rendering temperature	Steam pressure	Heating period	Lipid content of raw fat	Moisture content of lard	Yield[f]
		° F.	*° F.*	*p.s.i.g.*	*minutes*	%	%	%
J	22 manual	47	203	50	8	87.9	0.32	90.9
	23 mechanical	47	203	50	8	87.7	0.23	92.0
K	24 manual	26	203	50	8	90.0	0.25	90.5
	25 mechanical	26	203	50	8	89.3	0.28	96.8

[f] Based on lipid content of raw fat.

water-jacketed drum and slowly stirred for 15 minutes; the lard and tissue were cooled to 140° F. (60° C.) and separated by filtration. The remaining half of the batch was discharged into a galvanized iron drum and slowly stirred for 15 minutes; this portion cooled to only 185° F. (85° C.) and was also separated into lard and tissue fractions by filtration. The moisture contents of the lard from the batches cooled to 140° and 185° F. (60° and 85° C.) were found to be 0.14 and 0.28%, respectively.

The results show that appreciable absorption of moisture occurred, particularly at the lower temperature, and indicate that cooling and slowly stirring batches prior to fractionation by filtration is a potentially useful method of removing excess moisture from the lard.

Composition of residues. The composition of residues of connective tissue and incompletely rendered fatty tissue varied to some extent depending on variations in raw materials and yields. In rendering cutting fats with an average yield of 96.0%, residues containing an average of 43.2% moisture and 29.6% lipids were obtained; in rendering leaf fats with yields of 98.6% (average), residues containing an average of 55.8% moisture and 15.1% lipids were obtained.

Color and stability of lard. The Lovibond color of 10 samples of lard rendered at 203° F. (95° C.) ranged from 1 Yellow and 0.1 Red to 4 Yellow and 0.4 Red. The average was 2 Yellow and 0.2 Red, which denotes a very light color.

Each of 5 lots of cutting fats was separated into 2 batches, one of which was rendered at a maximum temperature of 203° F. (95° C.) and the other at 240° F. (115.5° C.) by the open kettle process. The stability of the samples rendered at 203° F. (95° C.), 6.5, 6, 4, 7 and 7 hours, and the stability of corresponding batches of open kettle lard, 6.5, 6.5, 3,5, 8.5 and 7 hours, A.O.M., were approximately equal. Since lard produced by the open kettle method is generally recognized to be relatively resistant to rancidification, and the data indicate that lard rendered at low temperatures is equally stable, the latter product had satisfactory though not exceptional stability.

Odor and flavor of lard.[k] A comparison of the intensity of odor and of flavor among 3 samples of experimental low temperature lard, 1 of commercial animal-vegetable fat shortening, 2 of commercial vegetable fat shortenings, and 5 of commercial lards, was made by an organoleptic test panel composed of 8 or 9 experienced judges. The paired test method was used. Separate samples of each product at room temperature were presented to the judges for odor and flavor evaluation. The judges scored a series of test pairs first for odor and then for odor and flavor combined. Each series of tests was repeated 3 to 4 hours later on the same day. A compilation of the results of the tests is shown in Table 6.

The results of the tests indicated that the intensity of the odor and flavor of lard rendered at low temperatures was low compared with that of samples of commercial lard. Furthermore, the odor and flavor of the low-temperature lard were milder than those of 2 samples of commercial vegetable fat shortening, although not so mild as the commercial animal-vegetable fat shortening, which was definitely bland.

[k] The organoleptic tests were conducted by C. Edith Weir, Animal Husbandry Division, Bureau of Animal Industry.

SUMMARY

Data and other information have been presented on the factors affecting the yield and quality of lard produced by a modified open-kettle process operated on a pilot plant scale in which the skin-free fatty tissue was ground, heated with agitation at relatively low temperatures, and filtered.

Under the optimum conditions studied, yields, based on the amount of lipids present in the raw fat, averaged 96.0 and 98.0% in rendering cutting and leaf fats, respectively. In some instances yields were increased by subjecting residues to centrifugation or to further disintegration by mechanical means.

The results show that the degree of comminution obtained in the grinding operation markedly affected yields. The yields were affected by variations in both the size of the openings through which tissue was ground and by variations in the temperature of the fatty tissue ground. The highest yields reported were obtained in rendering tissue which had been cooled to 0° F. (—17.8° C.) and ground through an 0.125-inch plate. Variations in the rate of heating during rendering and the temperature of rendering, over the ranges studied, were not found to be critical.

The production of lard having an acceptably low

TABLE 6

Comparison of the odor and flavor of low temperature-rendered lard, commercial lard, and other shortenings

Test pairs		Number of judgments	Odor			Flavor		
			Scores for A			Scores for A		
A	B		More intense than B	Less intense than B	No preference[l]	More intense than B	Less intense than B	No preference[l]
			%	%	%	%	%	%
Low temperature lard[m]	Commercial lards[n]	120	0	91.7	8.3	0	91.7	8.3
Low temperature lard	Vegetable fat shortenings[o]	68	11.8	50.0	38.2	8.8	73.5	17.7
Low temperature lard	Animal-vegetable fat shortening[o]	52	38.5	11.5	50.0	42.3	15.4	42.3
Animal-vegetable fat shortening	Vegetable fat shortening	16	0	62.5	37.5	0	37.5	62.5
Commercial lards	Vegetable fat shortening	120	81.8	5.0	13.2	78.4	0	21.6
Commercial lards	Animal-vegetable fat shortening	16	100.0	0	0	100.0	0	0

[l] Includes judgments when judges indicated that no difference was detected and those in which judges had different opinions in the first and second tests.
[m] The samples of low temperature lard were rendered by the procedure described in Series 2.
[n] Samples of 3 steam, 1 dry, and 1 open kettle rendered lards were included in commercial lards tested.
[o] The 2 vegetable fat shortenings and the 1 animal-vegetable fat shortening tested were nationally known brands of premium quality.

moisture content was possible provided a period of contact between lard and the proteinaceous residue was maintained, particularly at reduced temperature.

Lard rendered at low temperatures had a very light color, averaging 2.0 Yellow and 0.2 Red on the Lovibond scale. The lard had a much less pronounced odor and flavor than ordinary commercial lard, and was comparable to deodorized shortenings procured at markets. The average stability of samples of the lard, 6 hours, A.O.M., was acceptable but not outstanding.

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Microbiology of Frozen Orange Concentrate. II. Factors Influencing the Survival of Microorganisms in Frozen Orange Concentrate

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Cold pressed orange peel oil exerted a toxic effect upon Streptococcus faecalis. The pH and organic acid molecule are, however, the most important factors influencing the survival of enteric organisms in frozen orange concentrate.

In a previous paper *(3)* it was demonstrated that stock cultures of *Escherichia coli, Salmonella typhosa,* and *Shigella paradysenteriae* inoculated into orange concentrate which was subsequently frozen, could not be recovered after 24 hours of storage at —17° C. *E. coli* from fecal suspensions also failed to survive 24 hours of freezing storage in concentrate although *Str. faecalis* organisms from stock cultures as well as feces remained viable for the 3-week experimental period.

A number of factors have been suggested as being primarily responsible for the lethal action of orange concentrate against certain enteric organisms. Many essential oils have been found to have germicidal activity and orange peel oil, which is unavoidably present in commercial concentrate, is believed by many to be the bactericidal agent present in the juice. Piacentini *(7)* tested the disinfectant power of lemon and orange essences in aqueous solution against spore bearing organisms and found that the inhibitory power of both was greater than that of phenol.

It is well established that an increase in hydrogen ion concentration inhibits the growth and may even destroy microorganisms. In addition, organic acids are believed to exert a bactericidal effect greater than that provided by the hydrogen ion activity alone. However, the presence of sugar may influence the germicidal activity of acids. Erickson and Fabian *(2)* observed that the percentage of acid required to bring about a germicidal action on bacteria and yeasts was reduced slightly when combined with a fixed percentage of sugar. McFarlane *(5)* inoculated suspensions of *E. coli* containing 3.8 to 8.4 million cells per ml. into single strength orange juice and orange juice-sucrose solutions which were then frozen and stored at —17° C. In the unsweetened juice 97% of the organisms were destroyed within 48 hours while large numbers of cells were still viable after 26 weeks of storage in juice containing 40 to 50% sucrose. Shillinglaw and Levine *(8)* exposed *E. coli* to citric acid and found that in 0.02 *N.* citric acid 57.5 hours were required to kill 99.99% of the *E. coli* cells at 30° C. while the addition of 10% sucrose reduced the killing time to 32 hours.

Freezing temperatures while not necessarily lethal to microorganisms may markedly influence the action of deleterious agents present. Beard and Cleary *(1)* reported that there is an appreciable inhibition of the bactericidal effect of the hydrogen ion by low temperatures.

EXPERIMENTAL PROCEDURE AND RESULTS

To determine whether or not orange peel oil in concentrate has any effect on microbial survival, juice was carefully extracted from fresh oranges so as to avoid expressing any oil, and then concentrated under reduced pressure at 20° C. until 75% of the water was removed. After pasteurization, 9-ml. portions of the concentrate were pipetted into sterile tubes and inoculated with suspensions of the test organisms. Pure cold-pressed orange peel oil was then added in varying amounts and the tubes were agitated and quick-frozen at —17° C. At 24 hour intervals, tubes of the juice were thawed in cold running water, neutralized with 1 *N.* NaOH and plated to determine the number of surviving organisms. Plates were incubated a minimum of 24 hours before counts were made.

[a] Present address: Rexall Drug Co., Los Angeles, California.

TABLE 1

Effect of orange peel oil on survival of enteric organisms[a] in frozen orange concentrate stored at —17° C.

Organism	Storage time in hours	Plate count per ml.						
		Parts per million of oil in concentrate						
		No oil	100,000	10,000	1,000	100	10	1
Escherichia coli	24	0	0	0	0	0	0	0
Salmonella typhosa	24	0	0	0	0	0	0	0
Sh. paradysenteriae	24	0	0	0	0	0	0	0
Str. faecalis	24	394	0	0	0	31	158	335
Str. faecalis	48	110	0	0	0	25	20	160
Str. faecalis	72	98	0	0	0	4	45	112

[a] Initial number: Approximately 5,000 organisms per ml. of orange concentrate.

The results are shown in Table 1. Since *E. coli*, *Sh. paradysenteriae*, and *S. typhosa* were destroyed in the control containing no peel oil, conclusions cannot be drawn as to the effect of the oil on these organisms. However, *Str. faecalis* was destroyed by concentrations of peel oil of 1000 p.p.m. or greater and was adversely affected in a decreasing manner by 100 p.p.m. and 10 p.p.m. indicating the toxic action of the oil on this organism. Peel oil present in concentrations of 1 p.p.m. had no apparent effect on *Str. faecalis*.

In order to measure the simultaneous effect of citric acid and sucrose and freezing on enteric microorganisms, stock strains of the test organisms and dilute suspensions of fecal material were inoculated into solutions of citric acid and sucrose in concentrations approximating those found in orange concentrate. The suspensions were frozen at —17° C. and samples were thawed and examined at 24 hour intervals. Initial counts of the fecal suspensions were made on EMB and SF agars. Colonies which appeared on EMB agar were not identified although most showed the characteristics typical of coliforms on that medium. Colonies which grew on SF agar were identical to *Str. faecalis* colonies on that medium. It is evident from Table 2 and 3 that 3.8% citric acid is highly lethal to enteric microorganisms since none of the inoculated cells could be recovered by plating after 24 hours of freezing storage. Twenty per cent sucrose appeared to offer some protective action to all of the organisms against the detrimental effect of freezing. While sucrose had no effect in lessening the bactericidal action of citric acid on the gram negative cells, it did have some protective influence on *Str. faecalis* organisms both from the pure culture and from the fecal suspension.

TABLE 2

Survival of enteric organisms in distilled water, 20% sucrose, 3.8% citric acid, and 20% sucrose plus 3.8% citric acid stored at —17° C.

Organism	Plate count per ml.			
	Storage time in hours			
	0	24	48	72
Distilled water (pH 6.4)				
Escherichia coli	521,000	85,000	60,000	54,000
Salmonella typhosa	501,000	3,500	600	0
Sh. paradysenteriae	429,000	7,000	800	0
Str. faecalis	398,000	135,000	130,000	98,000
20% Sucrose (pH 6.4)				
Escherichia coli	521,000	68,000	45,000	38,000
Salmonella typhosa	501,000	4,250	0	0
Sh. paradysenteriae	429,000	2,600	400	0
Str. faecalis	398,000	225,000	190,000	110,000
3.8% Citric acid (pH 2.3)				
Escherichia coli	521,000	0	0	0
Salmonella typhosa	501,000	0	0	0
Sh. paradysenteriae	429,000	0	0	0
Str. faecalis	398,000	0	0	0
20% Sucrose plus 3.8% citric acid (pH 2.3)				
Escherichia coli	521,000	0	0	0
Salmonella typhosa	501,000	0	0	0
Sh. paradysenteriae	429,000	0	0	0
Str. faecalis	398,000	1,800	290	0

TABLE 3

Survival of fecal organisms in distilled water, 20% sucrose, 3.8% citric acid, and 20% sucrose plus 3.8% citric acid stored at —17° C.

Medium	Plate count per ml.			
	Storage time in hours			
	0	24	48	72
Distilled water (pH 6.4)				
EMB Agar	2,100,000	260,000	80,000	73,000
SF Agar	230,000	20,000	14,000	15,000
20% Sucrose (pH 6.4)				
EMB Agar	2,100,000	220,000	68,000	62,000
SF Agar	230,000	25,000	15,000	18,000
3.8% Citric acid (pH 2.3)				
EMB Agar	2,100,000	0	0	0
SF Agar	230,000	0	0	0
20% Sucrose plus 3.8% citric acid (pH 2.3)				
EMB Agar	2,100,000	0	0	0
SF Agar	230,000	900	103	0

Since the pH (2.3) of the citric acid solutions used in these experiments was lower than that normally found in orange concentrate (3.2) fecal suspensions were inoculated into citric acid solutions adjusted to a higher pH range with 1 *N*. NaOH. In this way the citrate ion remained constant but the hydrogen ion was variable. The results (Table 4) indicate that citric acid is lethal to coliforms at the pH and concentration usually found in orange concentrate. The bactericidal action of the acid on the enterococci appears to be proportional to the hydrogen ion concentration.

The experimental results seemed to indicate that acidity of orange concentrate was the important factor contributing to the destruction of certain of the organisms in pure culture and the coliforms of fecal material. A study was made to determine whether the citric acid molecule exerted a more toxic action on microorganisms frozen in solution than could be accounted for by the hydrogen ion alone. Two series of tubes were adjusted to the identical pH range of 2.2 to 6.0. One set contained 3.8% citric acid and varying amounts of NaOH. The second set contained distilled water adjusted to a similar pH range with HCl. Each

TABLE 4

Survival of fecal organisms[d] in citric acid solutions of varying pH stored at —17° C.

Medium	Storage time in hours	Count per ml.			
		Distilled water pH 6.7	Citric acid		
			pH 3.8	pH 3.5	pH 3.2
EMB	24	1,240	0	0	0
SF	24	715	119	134	11
EMB	48	718	0	0	0
SF	48	140	86	16	0
EMB	72	509	0	0	0
SF	72	37	0	0	0

[d] Initial number: EMB 39,000, SF 11,000.

tube was inoculated with an aqueous suspension of a pure culture of *E. coli*. To determine the effect of subzero temperature on the bactericidal action of the acid half of the tubes were stored at 4° C. and the rest at —17° C. In 24 hours plate counts were made on nutrient agar.

TABLE 5

Effect of varying pH and temperature on survival of E. coli[e]

pH	Plate count per ml. after 24 hours			
	At 4° C.		At —17° C.	
	Citric acid	Hydrochloric acid	Citric acid	Hydrochloric acid
2.2	0	0	0	0
2.4	0	0	0	0
2.6	0	100	0	0
2.8	0	190	0	0
3.0	0	300	0	0
3.2	0	470	0	0
3.4	0	800	0	0
3.6	0	1,100	0	0
4.0	90	16,000	0	0
4.2	800	14,000	0	8
4.4	6,500	18,000	160	200
4.8	7,200	21,000	900	400
5.0	9,500	22,000	700	1,200
5.2	9,800	26,000	800	900
5.4	8,200	24,000	1,100	1,900
5.6	13,200	26,000	1,400	3,100
5.8	19,000	29,000	2,600	3,800
6.0	21,000	33,800	3,400	4,600

[e] Initial number: 60,000 cells per ml.

It is apparent from Table 5 that at 4° C. the bactericidal action of citric acid is markedly greater than HCl at comparable hydrogen ion concentrations. Whether the difference is the result of osmotic pressure has not been determined. At —17° C. there is little difference in toxicity between the 2 acids but the deleterious effect of freezing is quite evident. The results in regard to the greater toxicity of citric acid as compared to HCl are in agreement with the conclusions arrived at by Nunheimer and Fabian *(6)*, Levine and Fellers *(4)*, and others who showed that organic acids exert a more toxic effect than would be indicated by their degree of dissociation. It is apparent from the results that the lethal action of the hydrogen ion is augmented by freezing rather than diminished.

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Microbiology of Frozen Orange Concentrate. III. Studies of Enterococci in Frozen Concentrated Orange Juice[a]

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Enterococci were found in all samples of commercially packed frozen orange juice concentrate obtained from 3 processing plants after 2 to 8 months of storage. The organisms were identified as Streptococcus faecalis and Streptococcus liquefaciens. The significance of the presence of these organisms in orange juice can not be determined until more is known about their natural habitat and the sources of contamination.

Pollution of frozen orange juice is now determined by the presence of coliform bacteria, particularly *Escherichia coli*. Members of the genus *Aerobacter* have limited sanitary significance in foods of vegetable origin as these organisms are widely distributed in vegetation and soil. The presence of *E. coli*, which is a common inhabitant of the intestinal tract of man, is a more significant indication of fecal pollution.

To be considered as a satisfactory indicator of fecal pollution, it is necessary that the organism used outlive the enteric pathogens. Evidence regarding the survival

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of coliforms in frozen orange juice is at present conflicting. Pure culture studies by Schrader and Johnson *(21)* and Martinez *(14)* in single strength juice and studies of fecally contaminated concentrate by Appleman and Hahn *(1)* have indicated that coliforms remain viable for relatively short periods while examinations of experimental packs by Wolford and Berry *(27)* and commercial packs by Wolford *(28)* have revealed viable coliforms after relatively long periods of storage.

Intestinal pathogens inoculated in orange juice have been found to be viable after 1 to 7 days, depending upon the organism, size of inoculum, temperature of storage, and whether single strength or concentrated juice was used, Douglas *(7)*, Beard and Cleary *(3)*, Duncan, Douli, Miller, and Bancroft *(8)*, Martinez *(14)*, and Appleman and Hahn *(1)*. It is possible, however, that under commercial conditions intestinal pathogens may also survive for longer periods than is indicated by these laboratory studies.

Elrod *(9)* pointed out that some organisms belonging to the genus *Erwinia*, which are plant pathogens, may be confused with *Escherichia-Aerobacter* organisms. If certain of these organisms giving IMVIC reactions of *E. coli* lose their ability to decompose pectin they become indistinguishable from *E. coli*. Thus *E. coli*-like organisms isolated from orange juice may not be of fecal origin.

It is possible that *E. coli* is not the best indicator of fecal contamination of frozen orange juice.

Ostrolenk, Kramer, and Cleverdon *(18)* and Burton *(5)* have suggested the use of enterococci as indicators of fecal pollution of frozen foods since these organisms were found to be more resistant to low temperatures than the coliforms. The original purpose of this study was to investigate the possibility of using enterococci as indicators of pollution of orange juice. However, while studying the survival of coliforms and enterococci in fecally contaminated orange juice during the preliminary work, enterococcus-like organisms were found in several samples of uncontaminated commercially packed frozen orange concentrate. Consequently, the identification of these organisms by biochemical, cultural, and serological studies, and the determination of their prevalence in commercially packed frozen orange concentrate became important.

EXPERIMENTAL METHODS AND RESULTS

For the study of the prevalence of these organisms in this product, 42 cans of frozen orange juice concentrate were obtained from 3 processing plants in southern California. The samples were examined after storage at the plants for 2 to 8 months at approximately —10° C. Samples consisting of 50, 30, 20, 10, 1.0, and 0.1 ml. portions from each can were inoculated into phenol red lactose broth (Difco). The pH was adjusted by adding sterile NaOH until the original red color of the medium reappeared. The inoculated flasks and tubes were then incubated at 37° C. for 48 hours after which subcultures to eosin methylene blue agar (Difco) were made. After 48 to 72 hours at 37° C., colonies of enterococcus-like organisms obtained were punctiform, pink in color and often contained purple centers. Gram-stained preparations of these colonies revealed spherical to ovoid cells which occurred singly, in pairs and occasionally in short chains. They were gram positive, but decolorized readily so that many of them at times appeared to be gram negative. These organisms were isolated from all cans, and in most instances from the 0.1 ml. samples (Table 1).

TABLE 1

Distribution of Enterococci in frozen orange concentrate processed by 3 companies in Southern California

Company	No. of samples examined	Number of positive tests using 50 ml. to 0.1 ml. of juice as inoculum					
		50 ml.	30 ml.	20 ml.	10 ml.	1.0 ml.	0.1 ml.
A	6	6					
	12	12	11	12	12	11	11
B	8	8	7	8	6	8	8
	4	4	3	4	3	4	3
C	12	12	12	11	11	11	12

For the identification of the enterococcus organisms, the criteria as given by Bergey's Manual *(4)* were used. Seventy-six strains of these organisms, none of which were beta hemolytic, were examined. All strains grew at 10° C., survived 60° C. for 30 minutes and were able to grow at pH 9.6 and in the presence of 0.1% methylene blue. Only 73 strains grew at 45.5° C., 71 in the presence of 6.5% NaCl, and 63 liquefied gelatin. Consequently 63 strains are identified as *Streptococcus liquefaciens* and 13 as *Streptococcus faecalis*.

Serological studies of 7 strains were made at the Bacteriological Section of the Communicable Disease Center, Chamblee, Georgia, through the courtesy of Dr. Martin Frobisher, Jr. These studies proved them to be Lancefield group D streptococci.

Coliform organisms were isolated from 4 of the cans. The IMVIC patterns of the organisms from 3 of the cans were (− − + +) indicating *Aerobacter aerogenes* while the fourth can contained organisms giving a (+ + − −) reaction indicating *E. coli*.

Estimations of the number of enterococci in the concentrate were made by preparing dilutions ranging from 1:10 to 1:1,000,000. Then 5 replicate samples of each dilution were examined by inoculating 1.0 ml. portions into phenol red lactose broth (Difco) and incubating at 37° C. for 48 hours. The tubes were considered positive when subcultures to eosin methylene blue agar plates contained typical colonies which, upon gram strained preparation revealed typical organisms. The most probable number of enterococci was determined by using the table in *Standard Methods for the Examination of Water and Sewage (24)*. The 3 cans examined in this manner were found to contain 170, 540, and 1600 enterococci per ml. (Table 2).

TABLE 2

Most probable number of Enterococci in samples of frozen orange concentrate as determined by phenol red lactose broth inoculation and confirmation

Company	Number of tubes for each dilution	Number of positive tubes at each dilution						Most probable number per ml.
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
C	5	5	4	1	0	0	0	170
B	5	5	5	2	0	0	0	540
B	5	5	5	3	1	0	0	1600

DISCUSSION

The survival of enterococci at low temperatures after storage for long periods is in agreement with the reports of Dible *(6)*, Sherman *(22)*, and others as far as resistance of this group of organisms to low temperatures is concerned. The survival of coliforms in commercially packed orange juice after long periods of storage has also been found by Wolford *(28)*. On the other hand, pure culture studies and studies of fecally contaminated juice indicate that coliforms remain viable for short periods, as noted by Schrader and Johnson *(21)*, Martinez *(14)*, and Appleman and Hahn *(1)*.

Enterococci have been reported in various other foods. Early investigators have found them in milk; McFarlane and Colesnick *(16)* and Solowey and Watson *(23)*

in egg powder; Ostrolenk and Hunter *(17)* in fresh frozen vegetables; Burton *(5)* in frozen vegetables and cantaloupe; and McCleskey and Boyd *(15)* in crabmeat. To this list may now be added frozen orange juice.

The presence of considerable numbers of enterococci in frozen orange juice is evidently not a peculiarity of any one particular plant but was found in the product of three different processing plants. Furthermore, these studies include examinations of the products of 2 seasons. To determine the source of these organisms would require bacteriological examinations of each step in the process from the fruit and its environment in the grove to the packed and frozen juice.

Several possible sources must be considered. One of these is direct contamination of the fruit or its juice by fecal material since these organisms are believed to be common inhabitants of the intestinal tract of man and warm blooded animals. That this type of contamination occurs as often as would be indicated by the frequency of their presence in the juice does not seem likely, even taking into account contamination of the fruit by birds.

Contamination by soil is another possibility. Winter and Sandholzer *(25, 26)* found enterococci in pasture soils but not in virgin soils or soils from wooded areas. Enterococci according to Mallman and Litsky *(13)* can survive in soil for varying lengths of time depending upon the type of soil and organic content. Contamination from soil may thus indicate pollution but not necessarily of recent origin. Contamination by water, on the other hand, may indicate more recent pollution since these organisms will not survive for long periods in fresh water, as noted by Greer *(11)*, Prescott, Winslow, and McCrady *(19)*, and others.

That enterococci have been isolated from plants has been pointed out by Sherman *(22)* and it may be that here is another natural habitat of these organisms. Sanderson and Fitzgerald *(20)* and Fitzgerald *(10)* have reported the isolation of *S. faecalis* from peas in the pod and from kernels of corn while Kuehner *(12)* isolated several strains of enterococci from aseptically peeled cucumbers and squash.

Sherman *(22)* states that *S. faecalis* is the most abundant streptococcus in the human intestine. He notes that the source of *S. liquefaciens*, which differs from the former by its ability to liquefy gelatin, is probably also in the intestine of man and warm blooded animals. Both occur rather commonly on plants but some strains of *S. liquefaciens* from plants are more actively proteolytic than are the characteristic types obtained from stools and milk. Bagger *(2)* noted that of enterococci from various human sources, only 10% were gelatin liquefiers. Burton *(5)* reported that of 262 strains of enterococci isolated from frozen vegetables and cantaloupe, 86% were *S. faecalis* while 14% were *S. liquefaciens*. However, it was noted that of the 76 strains from orange juice, only 17% were *S. faecalis* while 73% were *S. liquefaciens*. This difference in the predominating species may indicate that the enterococci in orange juice are not primarily of fecal origin. On the other hand, it may also indicate that *S. liquefaciens* is more resistant to the adverse conditions found in the frozen orange concentrate.

It is therefore apparent that from a public health standpoint the significance of the presence of enterococci in orange juice can not be determined until more is known about their natural habitat and the sources of contamination. Because of their survival in frozen concentrated orange juice for prolonged periods, they may be superior to the coliforms as indicators of sound production practices.

SUMMARY

While studying the survival of coliform and enterococcus organisms in fecally contaminated orange juice, gram positive cocci, later identified as enterococci, were isolated from several uncontaminated control samples of juice. To study the occurrence of these organisms, forty-two cans of commercially packed frozen concentrated orange juice from 3 processing plants were examined after storage at —10° C. for periods ranging from two to eight months. Of 76 strains of enterococci isolated, 63 were identified as *Streptococcus liquefaciens* and 13 as *Streptococcus faecalis*. Serological studies of seven strains proved them to be Lancefield Group D streptococci. Enterococci were isolated from all cans, even in 0.1 ml. samples. M.P.N. determinations of enterococci in juice from 3 of the cans resulted in 170, 540, and 1600 per ml. The enterococci are considered more resistant to adverse environmental conditions than *Escherichia coli* and thus in recent years these have been suggested as indicators of pollution of frozen foods. Since the enterococci were found consistently in frozen concentrated orange juice, and since the source has not been definitely determined, the significance of their presence is not established.

Coliform bacteria were isolated from 4 of the cans.

Acknowledgment

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Structural Changes Occurring in Muscle Tissue During Repeated Freezing and Thawing[a]

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Various histological studies have revealed some important facts concerning the behavior of muscle tissue of meat animals after slaughter. The effect of repeated freezing and defrosting of pork and beef muscles is presented. The changes occurring in several selected histological features is illustrated and discussed.

Various histological studies have revealed some important facts concerning the behavior of muscle tissue of meat animals after slaughter. In this study some histological changes which occurred as a result of repeated freezing and thawing of muscle tissue are reported. The longissimus dorsi muscle of both pork and beef was used, and after being submitted to the several phases of freezing and defrosting it was examined microscopically in each instance for detailed changes.

In an early histological study of frozen meat, Richardson and Scherubel *(4)* reported that ice crystals as they form around muscle cells must press against the separating cell walls, and such pressure exerted against a delicate membrane may possibly cause abrasions and ruptures. The sarcolema as it is frozen and thawed becomes permeable to proteins and they are thereby pressed out with the juice. The principal changes were reported to occur during the period of freezing, with only minor changes taking place as the meat aged in the freezer. In another early study Pennington *(3)* observed that cross striations were more distinct in muscle which had been frozen at 14° F. (—10° C.) for 1 month than in fresh sections. She also observed a slight separation of muscle bundles and early degeneration.

In a later study Shrewsbury et al. *(5)* observed that the shrinkage of muscle fibers is associated with the storage interval. Individual fibers were noted to become more condensed as the storage interval increased. Detailed observations of the stored meat indicated a progessive breakdown of the muscle tissue. Moran and Hale

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(2) found that there was less drip from frozen beef muscle stored at 14° F. (—10° C.) at the end of a 7-day period than there was at the end of a 145-day freezing period. Muscle globin loses part of its power to hold water, probably because it undergoes some form of denaturation by the action of the concentrated salt solution in the partly frozen muscle.

Hiner, Madsen, and Hankins *(1)* reported that when water freezes in muscle fibers, it crystallizes as pure water and pushes aside the substances which are dissolved or suspended in it. Slow freezing at 18° F. (—7.8° C.) causes ice crystal formation between the fibers. The water forming these crystals is drawn from juices within the fibers, leaving them in a semi-dehydrated condition and formed in irregular groups. As the temperature was lowered to —114° F. (—81.1° C.), the size of the ice crystals and ice areas between fibers decreased, fiber wall damage increased, and drip loss decreased when the meat was defrosted.

MATERIALS AND METHODS

The pork used in this study was obtained from the carcass of a hog which was raised, fattened, and slaughtered at Kansas State College. The loins of this carcass were cut into 3 equal parts.

After each phase of freezing and defrosting, chops 1½ cm. in thickness were removed from each of the 6 parts, and samples for histological study were removed at the same place from each chop and placed in fixing solution. A total of 9 chops were removed from each of the 6 parts, then 2 cross sections and 2 longitudinal sections were mounted and studied from each sample, thus giving a total of 216 sections of pork.

The beef used for this study was obtained from the right loins of 2 Angus steers, one of which was a normal animal and the other a "double muscle." The "double muscle" type animal which is sometimes also known as "bottle thighed," is abnormally thick and full in the thighs, with deep grooves appearing between the muscles. The hindquarters appear to be very large when compared with a normal animal. The double muscle was obtained so that a histological comparison could be made with the normal when both had been subjected to identical freezing and defrosting processes. The steers were likewise fed and slaughtered at Kansas State College. After aging for 1 week in the cooler, the carcasses were cut into wholesale cuts, and 10 steaks 2½ cm. in thickness were removed from each of the 2 previously mentioned loins. Then 4 samples were removed for histological study from each steak in approximately the same region of the longissimus dorsi muscle after it was subjected to the several phases of freezing and thawing. As in the case of the pork muscle, 2 longitudinal and 2 cross sections were cut from each sample, thus giving a total of 320 sections of beef for histological study.

As previously stated, the object of this study was to observe histological changes taking place in muscle tissue as a result of repeated freezing and thawing. While controls are not mentioned specifically, the first phase of treatment listed for each commodity is considered as a control, in that the tissue sections of subsequent phases are compared with those of phase 1. The detailed treatment given to each of the muscle samples studied is shown in Tables 1 and 2.

As each sample was collected it was placed immediately in 10% formalin or Zenker's solution. Following fixation, the samples were dehydrated and hardened in alcohol, cleared in oil of cedar, embedded in Tissuemat, and then mounted on wooden blocks for sectioning. Sections 8 to 10 microns in thickness were cut from each cross and longitudinal section of the muscle tissue samples on a rotary microtome, and secured to slides by phenolized egg albumen. One set of slides was stained by the hematotxylin-eosin method, and duplicates were stained by the use of Weigert's stain, hematoxylin, and van Gieson's stain, in the order named. The purpose in following 2 different staining methods was to prepare the tissues for a more detailed study.

TABLE 1

Treatment of the 6 pork loin sections prior to obtaining the different samples for histological study. Samples were removed from the frozen loin sections and again after the sections had defrosted, so that each constituted a phase of treatment.

Phase	Total No. of days in freezer	Temperature of freezer	No. of days defrosting [b]	Temperature of defrost room	Total No. of times defrosted	Total No. of times refrozen
		° F.		° F.		
1	11	5	4	38	1	0
2	13	—20	0 [c]	....	1	1
3	13	—20	3	38	2	1
4	15	—28	0 [c]	....	2	2
5	15	—28	2	38	3	2
6	18	—28	0 [c]	....	3	3
7	18	—28	5	38	4	3
8	25	—25	0 [c]	....	4	4
9	25	—25	3	38	5	4

[b] Number of days between each freezing and defrosting process. When samples were taken without defrosting or without refreezing, it is so indicated.

[c] Cut frozen.

TABLE 2

Treatment of the steaks prior to obtaining the different samples of muscles for histological study.

Phase	Number of days		Temperature of		Total number of times	
	in freezer	defrosting	freezer	defrost room	defrosted	refrozen
			° F.	° F.		
1	0	0	not frozen	not frozen	0	0
2	95	1	0 to —5	38	1	0
3	101	4	0 to —5	38	2	1
4	106	2	0 to —5	45	3	2

All slides were thoroughly examined for possible changes in the various histological details listed in Tables 3 and 4. Gradations from 1 to 4 were used in measuring the degree of structural changes noted in these details for each slide, and the end result was obtained by taking an average which is recorded in Tables 3 and 4.

Most of the terms used to describe the histological details are self-explanatory. Natural fragmentation is the breaking or shattering of muscle fibers caused by aging, freezing, and thawing of the meat. An attempt was made to distinguish this type of fragmentation from the mechanical fragmentation which is caused by fixing and handling the tissues in the laboratory. The term "vacuoles" describes small openings which are caused as a result of ice crystal formation and the subsequent melting of the ice particles. The tissue fluid observed was found between the muscle fibers.

OBSERVATIONS AND DISCUSSION

Pork. It was intended during this study to subject the pork tissue to more severe treatment than that which would be found under ordinary field conditions. Chops removed during the last phase were soft and mushy. From 10 to 50 ml. of drip loss was observed after the end of each defrosting period, depending on the amount of meat involved. The volume of drip loss became greater in proportion to the amount of meat as the experiment progressed.

The microscopic study of the pork revealed that the occasional loss of striations was associated frequently with disintegration of the muscle fibers. This was particularly true of the cross striations. An increase in the number of swollen fibers as illustrated in Figure 1 was noted as the study progressed. The fact that they took lighter stain indicated that they may be undergoing a

TABLE 3

Composite values for each phase of treatment of the pork

Points observed	Phase numbers									Gradations
	1	2	3	4	5	6	7	8	9	
Cross striations	1.00	1.00	1.16	1.00	1.16	1.83	1.00	1.00	1.00	Distinct 1 — 4 Indistinct
Longitudinal striations	1.00	1.00	1.00	1.00	1.00	1.50	1.00	1.00	1.00	Distinct 1 — 4 Indistinct
Swelling of fibers	3.00	3.00	2.16	2.16	2.50	2.50	2.50	2.83	2.16	Abundant 1 — 4 Absent
Natural fragmentation	3.83	3.83	3.00	3.00	3.00	2.83	3.00	3.00	2.83	Abundant 1 — 4 Absent
Mechanical fragmentation	1.66	2.00	2.16	2.66	2.16	2.50	2.00	2.16	1.83	Abundant 1 — 4 Absent
Rupture of sarcolema	3.83	3.83	3.00	3.00	3.33	3.16	3.00	3.00	3.00	Abundant 1 — 4 Absent
Density of col. fibers	2.50	3.00	2.50	2.66	2.66	3.00	2.33	2.66	2.50	Loose 1 — 4 Dense
Waves in col. fibers	2.66	2.83	2.83	2.16	2.83	2.66	3.50	2.66	2.33	Abundant 1 — 4 Absent
Intracellular vacuoles	4.00	3.50	3.66	3.83	3.16	3.33	3.16	3.33	3.00	Abundant 1 — 4 Absent
Intercellular vacuoles	4.00	4.00	4.00	4.00	3.00	3.00	3.16	3.16	3.00	Abundant 1 — 4 Absent
Granular disintegration	3.83	3.83	3.00	3.00	3.00	2.83	3.00	3.00	2.83	Abundant 1 — 4 Absent
U Waves	2.83	2.66	2.66	2.66	3.00	3.00	3.16	3.16	2.66	Abundant 1 — 4 Absent
V Waves	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	3.33	Abundant 1 — 4 Absent
Fibrillar waves	3.16	3.00	2.83	2.50	3.00	3.33	3.16	3.00	3.00	Abundant 1 — 4 Absent
Cohnheim areas	2.20	1.66	1.66	1.00	1.66	2.00	2.00	1.00	1.00	Distinct 1 — 4 Indistinct
Rigor ridges	3.83	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	Abundant 1 — 4 Absent
Tissue fluid	4.00	4.00	4.00	4.00	4.00	4.00	3.33	3.33	3.50	Abundant 1 — 4 Absent

retrograde process. Cohnheim areas and striations were indistinct in the swollen fibers.

An increase in granular material and a loss of striations invariably was associated with natural breaks in the muscle fibers. This type of fragmentation increased as the study progressed, while mechanical fragmentation followed no definite pattern. Breaks in the sarcolema increased in number during the latter phases of treatment, and were frequently associated with disintegration and breaking of fibers. These ruptures were associated with the collection of tissue fluid between the fibers.

Observations of the collagenous connective tissue were made for density and waviness. The density varied slightly from one phase to another but the end result proved that there was no significant change. The numerical ratings for the waves indicated that the changes in this feature were just about as insignificant as the density. The reaction to staining was normal in all phases.

Vacuoles which may be considered the result of ice crystal formation gave some indication of the amount of this type of freezing in the tissues. There was an

TABLE 4

Composite values for each phase of treatment of the beef

Points observed	Animal	Phase numbers				Gradations
		1	2	3	4	
Cross striations	Normal	1.00	1.50	1.30	1.00	Distinct 1 — 4 Indistinct
	Double muscle	1.50	1.70	2.11	2.25	
Longitudinal striations	Normal	1.00	1.00	1.00	1.00	Distinct 1 — 4 Indistinct
	Double muscle	1.00	1.10	1.00	1.00	
Swelling of fibers	Normal	3.89	3.30	3.00	3.00	Abundant 1 — 4 Absent
	Double muscle	3.22	2.70	2.30	1.66	
Natural fragmentation	Normal	3.22	3.30	3.00	3.00	Abundant 1 — 4 Absent
	Double muscle	3.00	2.70	2.30	1.66	
Mechanical fragmentation	Normal	3.00	2.30	2.60	1.80	Abundant 1 — 4 Absent
	Double muscle	2.88	2.40	2.30	2.50	
Rupture of sarcolema	Normal	3.77	3.40	3.33	3.40	Abundant 1 — 4 Absent
	Double muscle	3.22	3.10	3.00	3.00	
Density of col. fibers	Normal	3.22	3.00	2.40	3.80	Loose 1 — 4 Dense
	Double muscle	2.44	2.70	2.40	2.33	
Waves in col. fibers	Normal	2.22	3.33	2.60	2.60	Abundant 1 — 4 Absent
	Double muscle	3.00	2.50	2.60	2.33	
Intracellular vacuoles	Normal	4.00	4.00	3.80	3.80	Abundant 1 — 4 Absent
	Double muscle	4.00	3.10	3.50	3.66	
Intercellular vacuoles	Normal	4.00	3.60	3.70	3.40	Abundant 1 — 4 Absent
	Double muscle	4.00	3.00	3.25	3.16	
Granular disintegration	Normal	3.33	3.33	3.00	3.00	Abundant 1 — 4 Absent
	Double muscle	3.11	2.70	2.20	1.66	
U waves	Normal	2.55	2.50	2.30	3.00	Abundant 1 — 4 Absent
	Double muscle	2.87	3.00	2.33	2.66	
V waves	Normal	3.44	3.20	2.40	3.60	Abundant 1 — 4 Absent
	Double muscle	3.75	3.60	2.55	3.33	
Fibrillar waves	Normal	3.44	3.20	3.80	4.00	Abundant 1 — 4 Absent
	Double muscle	3.87	3.20	3.11	3.50	
Cohnheim areas	Normal	1.22	1.33	1.00	1.00	Distinct 1 — 4 Indistinct
	Double muscle	1.11	1.40	1.33	1.25	
Rigor ridges	Normal	4.00	4.00	4.00	4.00	Abundant 1 — 4 Absent
	Double muscle	4.00	4.00	4.00	4.00	
Tissue fluid	Normal	4.00	3.90	3.60	4.00	Abundant 1 — 4 Absent
	Double muscle	4.00	3.80	3.70	3.66	

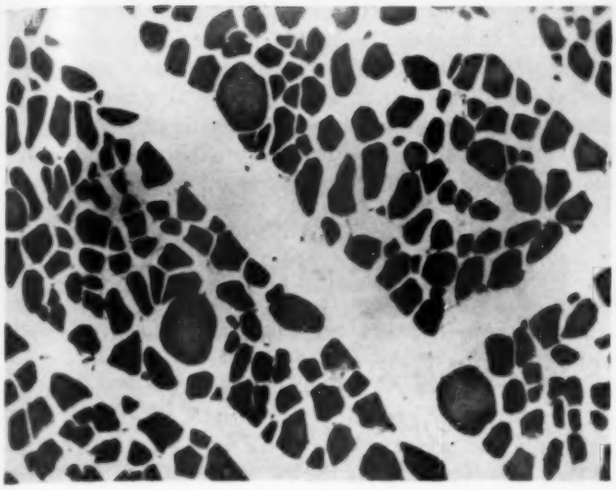

Figure 1. Cross section through sample of pork chop taken at end of third phase showing several large muscle fibers. It is believed that the large fibers were undergoing some sort of a retrograde process. × 125. (Courtesy Armed Forces Institute of Pathology—Kansas State College slide No. R-1-B.)

unsteady increase in both intracellular and intercellular vacuoles as the study progressed.

Granular disintegration increased as the study progressed. In the early phases small granular fragments were noted at the point of breaks in the fibers, and later small amounts of this type of degeneration spread to other parts of the cell. The granular disintegration could not be considered extensive at any time.

The frequency with which the *U* and fibrillar waves appeared was rather irregular, and there was not a great deal of variation from the first to the last phase. The *V* waves were almost entirely absent except for the last phase.

The Cohnheim areas observed in the cross sections became more distinct as the study progressed. It is expected that the sarcoplasm decreased as more fluid was withdrawn from the fibers during the latter phases of the defrosting and refreezing process. The decrease in the amount of sarcoplasm separating the fibrillae within the Cohnheim areas increased the density of the groups of fibrillae, thus causing the Cohnheim areas to stand out more distinctly.

No rigor nodes whatsoever were observed during the study. A few rigor ridges were seen in the sections of the first phase, but the remaining phases were entirely without ridges. A granular-appearing tissue fluid was observed between the muscle fibers in varying amounts during the last 3 phases of the study. Some slides displayed small amounts, and in others larger areas were formed. The presence of this fluid indicated that a gradual breakdown of muscle fibers was taking place. The tissue fluid is illustrated in Figure 2.

Beef. The beef did not undergo as many phases of freezing and defrosting as the pork, and the macroscopic appearance of the beef at the end of the last phase did not indicate that any great change had taken place. The drip loss did, however, increase toward the end of the study.

The longitudinal striations remained quite distinct in the muscle of both the normal and double muscled steers, but the cross striations became less distinct in the double muscle than in the normal as the study progressed. This would indicate that the degenerative changes were greater in the double muscle than in the normal.

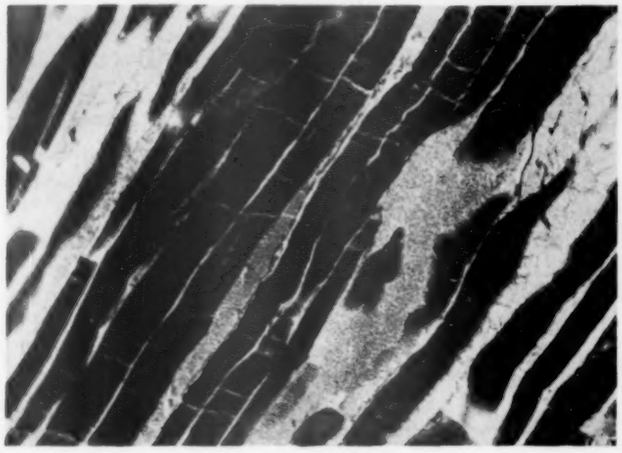

Figure 2. Longitudinal section through sample of pork chop taken at end of sixth phase showing tissue fluid between the muscle fibers. × 125. (Courtesy Armed Forces Institute of Pathology—Kanses State College slide No. R-1-E.)

Swollen fibers increased in number as the study progressed in both the normal and the double muscle, but the increase was greater in the double muscle. Natural fragmentation increased proportionately in the same manner. The mechanical fragmentation was irregular and therefore considered to be insignificant. Figure 3 shows that cross striations are relatively clear at the points of breaks in mechanical fragmentation. Ruptures in the sarcolema increased with each phase,

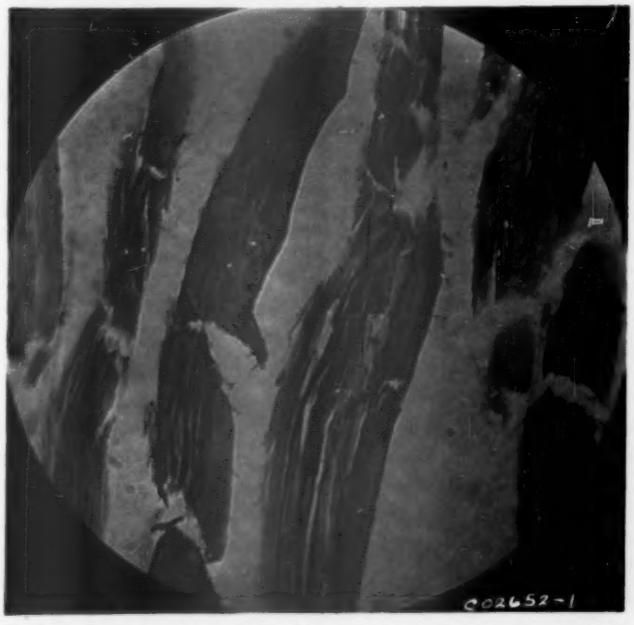

Figure 3. Longitudinal section of longissimus dorsi of steak No. 2 of the double muscle removed at the end of phase 2. Photo illustrates some sharp breaks which are called mechanical fragmentation. The cross striations of the large center fiber appear quite clearly even though the structure is shattered. (× 450.)

with a larger number of breaks noted in the double muscle.

Changes in the connective tissue were irregular in both muscles during the study. The lack of uniformity in the changes occurring in both the density and waviness of the connective tissue fibers made it impossible for any definite conclusions to be made regarding this histological feature.

Vacuoles were observed in both types of muscle during the latter phases of treatment, with more intracellular vacuoles present in the double muscle than in the normal. This would indicate that intrafibrillar freezing takes place more frequently in the double muscle.

Areas of granular disintegration usually appeared around the ends of broken fibers. As the study progressed the disintegration spread to other parts, and was associated with the loss of striations in the muscle fibers. This is illustrated in Figure 4. Granular disintegration was greater in the double muscle than in the normal. Inasmuch as the temperature and length of time was not favorable for a large amount of autolysis, it is believed that many of the changes noted were due to the repeated freezing and thawing.

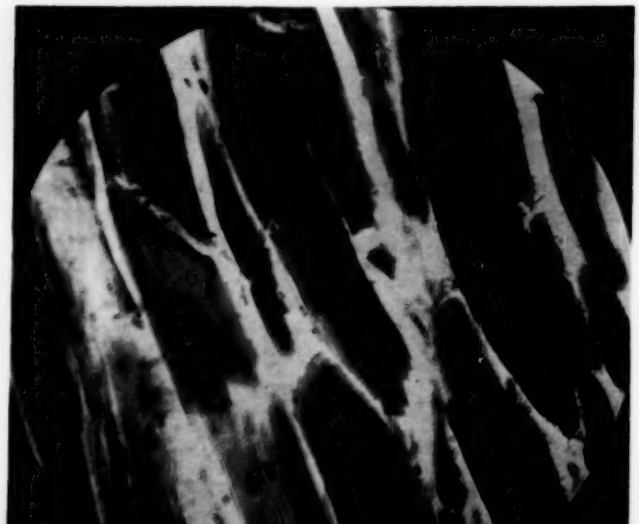

Figure 4. Longitudinal section of longissimus dorsi of steak No. 5 of the double muscle removed at the end of phase 2. Photo illustrates a small amount of disintegration at the ends of the broken fibers. Most of the cross striations have disappeared or are indistinct. (× 450.)

The amount of waviness in the muscle fibers varied irregularly from one phase to another in both the normal and the double muscle. No significance could be attached to the changes which were observed.

The changes occurring in the Cohnheim areas were likewise irregular, though they were slight. With a gradual dehydration of the tissues more distinct Cohnheim areas would be expected as the cross sections were examined. This was not always the case.

No tissue fluid was observed during the first phase in either the normal or the double muscle. A small amount was noted in each of the remaining phases except the last phase of treatment for the normal when no fluid was found. The greater amount of fluid observed in the double muscle indicated that the breakdown of the double muscle was a trifle faster than the normal.

SUMMARY AND CONCLUSIONS

Several changes occurred in the muscle tissue as a result of repeated freezing and thawing. Mechanical changes from fixation and the microtome knife were also observed.

The various degenerative processes noted during the study increased generally each time the meats were defrosted and refrozen.

It is believed that both the intracellular and intercellular ice crystal formation contributed to the fragmentation of the fibers.

The connective tissue remained about the same throughout the study, except for the changes in the epimysium where it formed the outer covering of the *longissimus dorsi* of the beef carcass. The staining tendencies of this tissue along with the small amount of shattering observed indicated that some degenerative changes were taking place during the last 2 phases of the study.

The freezing and thawing caused an increased amount of drip loss. As the number of broken muscle fibers increased, more fluid was released. This caused the Cohnheim areas to become more distinctly separated. A granular-like tissue fluid was found between the muscle fibers during the last phases of the study. Many muscle fibers presented a soft crumbled appearance at the broken ends after the muscles had undergone a series of defrosting and refreezing treatments.

It was quite evident that the muscle of the double muscled steer displayed more degeneration at the end of each phase of treatment than did the normal.

Acknowledgment

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Some Implications of Recent Theoretical Work on Canning Processes

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New approaches to the evaluation of canning processes have recently been suggested by Stumbo, Gillespy, and Hicks. The basic principles adopted by these workers are identical, and the differences in detail are not serious. These basic principles are outlined and their application to practical process calculations is discussed.

The heat treatment of canned foods must be sufficient to destroy organisms which could grow in the can and cause spoilage or endanger the health of consumers. The amount of heating required must, therefore, depend on the resistance to heat of the spores present in the pack, and on the rate of heat penetration into the contents of the can.

The methods of process evaluation in common use at present were worked out by Bigelow, Bohart, Richardson, and Ball *(3)* and by Ball *(1, 2)*. Some modifications in detail of the procedures recommended by these authors have been suggested from time to time but the general principles of their methods seem to have been unquestioned untnil 1948 when Stumbo published his first paper on the subject *(6)*. The appearance of this paper stimulated some others to develop ideas which they had been thinking about but had not worked out fully. As the result of this, 2 papers have appeared so far, one by Gillespy *(4)* and one by Hicks *(5)*. The basic principles of these two papers are almost, if not quite, identical. Between the preparation and publication of these papers a second paper by Stumbo *(7)* appeared in which virtually the same basic principles were expressed. I believe that at least one other worker has been developing similar ideas independently. The development of these ideas involves rather elaborate algebra and a somewhat intricate set of definitions and terminology. In these details the 3 papers are all different and all are difficult to read. It is almost inevitable that a discussion giving details of the algebraic methods will be difficult reading. In this note an attempt will be made to outline the basic ideas giving no more algebraic details than are absolutely necessary. The practical implication of the new ideas will also be discussed.

Classical methods of process evaluation. The classical methods were based on the concept of thermal death time of bacterial spores current at the time they were worked out. Experiments had shown that a certain time of heating at a particular temperature would destroy all the spores in a standard suspension in a standard medium, this time varying with the temperature. It was found that the relation between the thermal death time and the temperature of heating could be represented with high accuracy by an equation of form:

$$\tau = \tau_o e^{-b(T - T_o)}, \qquad (1)$$

where τ is the thermal death time at temperature T; τ_o is the thermal death time at a reference temperature, T_o; and b is a constant for a particular organism and medium.

In a can of food being processed the temperature varies with time and with the position in the can. Assuming that the obvious literal interpretation of thermal death time is legitimate, it is clearly only necessary to consider the slowest heating point of the can; if the heat treatment at that point is equivalent to heating for more than the thermal death time for the spores considered, all parts of the can must receive adequate heating. The classical methods of process evaluation, which are based on these assumptions, consist essentially in calculating the time at 250° F. (121.1° C.) which, from the point of view of the destruction of the bacterial spores, is equivalent to the actual heat process at the slowest heating point of the can. This equivalent time at 250° F. (121.1° C.) which is generally called the F value, is derived from data on the rate of heat penetration into the pack and from the temperature coefficient of the thermal death time, the constant b in equation (1). In the direct method of Bigelow et al *(3)*, the F value was evaluated graphically whereas Ball expressed it mathematically in terms of exponential integrals which have been tabulated.

Basic criticisms of classical methods. Stumbo *(6)* drew attention to experimental observations that the course of the destruction of bacterial spores by heat is logarithmic, that is, it can be represented by equations of the form:

$$(dn)/(dt) = -kn, \qquad (2)$$

or

$$n = n_o e^{-kt}, \qquad (3)$$

where n is the number of spores surviving at time t; n_o is the number of spores presents at time $t = 0$; and k is a constant for a particular organism and temperature of heating in a particular medium.

These equations could be expressed equally well in terms of concentrations of spores or numbers per unit volume of suspension. Stumbo's and later criticisms of the classical methods hinge on equation (3) and the interpretation of values of n which are less than 1.0. Clearly, only whole numbers of viable spores can exist but equations (2) and (3) are obviously statistical laws so that values of n less than 1.0 should be interpreted as probabilities of a spore surviving. Gillespy has produced experimental evidence that an equation of the form (3) is, in fact, valid down to values of n less than 0.1 when fractional values of n are interpreted as probabilities of a spore surviving. There can be no reasonable

doubt that this interpretation of fractional values of n is right; it might be doubted whether it is legitimate to extrapolate from the experimental data to the extent which is involved in using equation (3) for the calculation of values of n down to, say 10^{-12} but it is not feasible to obtain a direct experimental check of this. A fuller understanding of the mechanism of the destruction of spores by heat might lead to a modification of equation (2) and would certainly give greater confidence in extrapolating to very small values of n.

Stumbo *(6)* pointed out that the number of survivors (interpreted statistically as above) is proportional to the number of spores present initially. The volume of material, and the number of spores, subjected to the conditions at the slowest heating point of the can is very small. There are much larger regions, that is, larger numbers of spores, which receive more severe heat treatment than the center, but in which the chance of survival would be greater because of the larger initial number of spores. He therefore proposed that processes should be based on calculations of the chance of survival of a spore in the region of the can for which this is greatest.

Stumbo *(7)*, Gillespy *(4)*, and Hicks *(5)* went a stage further and suggested that the most logical procedure is to add up, or integrate, the chances of survival over the whole volume of the can.

Some definitions and terminology. In considering the sterilizing value of a process, two general types of numerical scale are in use, namely, that of number of survivors or some related quantity and that of equivalent time at 250° F. (F value) or some related quantity. The first of these is a linear scale in terms of number of survivors and the second is a logarithmic one. Both types of scale are likely to remain in use.

For the linear type of scale Hicks introduced the terms survival number for a can, survival ratio for a can, and survival ratio for a point in the can. The survival number for a can (n_t) is simply the number of survivors, interpreted statistically as already explained. The survival ratio for a can is the ratio n_t/C_o, C_o being the initial concentration of spores (number per unit volume). The survival ratio for a point is the ratio C_t/C_o, C_t being the final concentration of spores at that point. Gillespy expressed the same ideas by the terms sterility with respect to cans and sterility with respect to spores.

For the corresponding logarithmic scale most workers have followed Ball in using F values, that is, equivalent time at 250° F., in considering particular points in a can. Gillespy *(4)* has also used F values for whole cans. This practice has some advantages but it must be made quite clear whether the F value for the can corresponds to n_t/C_o or the average value of C_t/C_o, on the linear scale. The second of these alternatives may seem the more logical and was used by Gillespy, but then the F value for the can would not be a measure of the chance of the can being spoiled; this would depend also on the size of the can since one surviving spore can lead to spoilage.

Hicks also introduced the term equivalent volume (v) which is defined by the equation:

$$v = n_t/C_{t_o}, \qquad (4)$$

where C_{t_o} is the final concentration of spores at the slowest heating point. Thus the number of survivors in the can (interpreted statistically) is numerically equal to the number of survivors there would be in a volume v subjected to the conditions at the slowest heating point of the can.

Criteria for an adequate process. As already explained, it is not possible to determine a process which will destroy all the spores present initially; it can only be stated that a given process will reduce the survival number for the can (the chance of a spore surviving in it) to 10^{-3}, one chance in 1000; 10^{-12}, one chance in a trillion (million million); or some such value.

It is logical to define an adequate process as one which will reduce the survival number for a can to some specified value, given a certain initial concentration of spores. Stumbo *(7)*, Gillespy, and Hicks have each, explicitly or implicitly, adopted a definition of an adequate process which is equivalent to this but none of them has ventured an opinion as to what the specified value of n_t (or of n_t/C_o) should be. Present commercial practices based on Esty and Meyer's data for *Clostridium botulinum* and the classical process evaluation methods, amount to adopting a value of the order of 10^{-12} for the ratio n_t/C_o for many processes, but this is by no means a constant standard. It seems desirable that administrators, bacteriologists, and biometricians in the canning industry should be considering this matter carefully so that precise standards may be determined on a sound logical and experimental basis.

If n_t is considered as an independent quantity, its value should vary with the organisms under consideration. If those which could survive would cause spoilage but not endanger the health of consumers, such as flat sour organisms, a relatively large value of n_t would be appropriate, say 10^{-2} or 10^{-3}. If, however, there is risk of survival of highly toxic organisms such as *Cl. botulinum*, a very much lower value of n_t must be adopted. Whether this should be 10^{-6}, 10^{-12}, or 10^{-24} or some intermediate value is a question which should be faced by the executives in the canning industry.

Estimates of C_o might be obtained from bacteriological studies of material in canneries, but it would be difficult to obtain very precise values. This question is discussed briefly in the paper by Hicks *(5)*.

Methods of Process Evaluation Used by Stumbo, Gillespy, and Hicks.

Each of the workers carried out the calculations in 2 stages: a, calculating survival ratios or F values for particular points or small regions in the can; and b, integrating the survival ratios over the whole volume of the can. Stumbo used experimental heat penetration data for the heating phase and used Ball's formulas for the cooling phase. He considered a series of concentric cylindrical shells, equal small distances apart, calculated survival numbers for these by classical methods modified to take due account of the varying initial number of spores and carried out the second integration graphically. Stumbo's concentric cylinders are not bounded by isothermal surfaces so that his procedure cannot be exact. It seems unlikely, however, that the systematic

errors in his procedure would be of great practical importance. Stumbo considered both convection heating and conduction heating packs.

Gillespy has, so far, only published his work on solid packs. He considered a series of shells enclosing equal volumes and bounded by surfaces of equal F value. He did not discuss the geometry of these shells in detail. For the calculation of F values on these surfaces he used exact solutions of the equation of conduction of heat for the heating phase and an approximate solution of his own for the cooling phase. He found that the F values for these surfaces were approximately linear functions of the total volume inside them, over the range of volumes which it was necessary to consider. He therefore carried out the volume integration analytically.

Hicks used exact solutions of the equation of conduction of heat for both the heating and cooling phases in solid packs. He calculated F values and survival ratios for a series of points and evaluated the integral over the can volume graphically. He drew some conclusions about liquid packs from the consideration of the ideal case of a can heating and cooling uniformly and instantaneously.

Comparison of results obtained by Stumbo, Gillespy, and Hicks. A direct comparison of absolute values obtained by the three workers would be difficult to obtain and would be affected by their differences in treating the cooling phase. However, some approximate comparisons of relative values are easily obtained.

Stumbo concludes that center F values of 6.57 and 9.60 minutes would give equal survival numbers of the organism PA3679 in No. 10 size cans of conduction heating and convection heating products respectively. From either Stumbo's or Hicks' analysis the differences in these values must be almost independent of the absolute value over the range likely to be used in practice. Hicks' published figures were based on a different organism, but from Stumbo's data on the organism and Hicks' formulas and his Figure 2, it is easily shown that his analysis would indicate a difference of 3.45 min. compared with Stumbo's 3.03 min. On the survival number scale the discrepancy between Hicks' and Stumbo's results represents a factor of 2.6 which is not of great practical importance. Moreover, Hicks' figures for liquid packs are based on an ideal case and this would certainly lead to an overestimate of the equivalent volume, probably by a factor of the order of 2 or 3 on the survival number scale. The agreement between Stumbo and Hicks in relative values is, therefore, quite satisfactory.

Hicks gives complete calculations for the case of a No. 2½ can of a solid pack cooked 110 minutes at 240° F. (115.6° C.). He deduces that the F value at the center (F_c) is 7.05 minutes, and the equivalent volume is 13 ml. Using Gillespy's tables and formulas with Hicks' data, the values obtained are F_c, 6.77 min.; v, 8 ml. The discrepancy in F_c values is due to differences in the treatment of the cooling phase; this is discussed in the next section. The discrepancy in equivalent volumes is about 50% of Gillespy's value and no doubt represents a real difference in the approximation methods used. However, a factor of 1.5 in the survival number scale is quite negligible compared with the inevitable uncertainties in C_o and the desired value of n_f, that is, it is of no practical importance. The two workers agree that the equivalent volume is of the order of 1 to 2% of the volume of the can.

Treatment of the Cooling Phase

Ball, in his mathematical method, used an approximation for the cooling phase. The formulas and tables which he gave, based on this approximation, seem to have been widely used for packs of all sorts although he pointed out that the form of cooling curve he assumed was not always a good approximation to experimental curves. He suggested that, in such cases, the direct method or his arithmetical adaptation of it should be used. Gillespy's and Hicks' results agree in indicating that all products heating mainly by conduction should be included in the category of packs for which Ball's formulas will underestimate the sterilizing value of the cooling phase. This underestimation may be by a factor of 10^7 on the survival number scale so it is by no means negligible.

As already explained, Gillespy used approximate equations for the cooling phase whereas Hicks used exact equations but numerical approximations were made in applying them. The values quoted in the papers indicated that Gillespy's approximations led to rather lower estimates of the contribution of the cooling phase than those of Hicks. However, details of methods of calculating the cooling phase in packs for which the equation of conduction of heat applies exactly (with constant thermal properties) are likely to prove only of academic interest. There is a good deal of evidence in the records of this and other laboratories, that products which behave practically as pure conductors in the later stages of heating may not do so in the early stages of cooling; the initial cooling rates are often greater than the equation of conduction would indicate. These departures from the theoretical equations tend to be less with careful pressure cooling than when the pressure is not maintained with compressed air. The main cause of these departures is, no doubt, that the sudden thermal shock and pressure changes cause some circulation of the product inside the can. It would seem necessary, therefore, to base practical calculations on experimental cooling curves.

Practical Application of the New Ideas

The newer ideas have provided a logically satisfactory basis for process evaluation for one which was logically quite unsatisfactory. This is an important gain from the academic point of view. Further, this work should facilitate clear thinking about the criteria to be adopted and the relative importance of various factors affecting process values. This is likely to prove its most important practical consequence.

The new theories indicate that the estimates of the sterilizing value of processes made by the classical methods were too high. Furthermore, the difference is greater for products heating by convection than for those heating by conduction. There is, therefore, new scientific justification for the common practice of giving to

products heating by convection processes which appeared, from the classical evaluation methods, to be more severe than those used for conduction heating packs.

The increase in F_e, or F value at the center, over that indicated by the classical methods which is indicated as necessary by the new methods will be, for *Cl. botulinum*, about 0.7 minute for a No. 2 can and 0.9 minute for a No. 10 can of a liquid pack. These differences will vary little with the actual F_e value. For the organism PA3679 considered by Stumbo, the differences will be roughly 4 times as great. For a No. 2½ size can of a product heating by conduction the differences in F_e values indicated by the old and new methods will be of the order of 0.3 minute for *Cl. botulinum*.

Hicks investigated the variation of the equivalent volume of conduction heating packs with processing conditions. As was expected, it was found to vary, but only by a factor of the order of 2 for a wide range of processing conditions. The survival ratio for a can is proportional to the equivalent volume. This ratio will be 10^{-12} or less in practice; the initial concentration of spores can rarely be known within a factor of 100 or more and ideas as to the criteria which should be adopted will probably vary by an even greater factor. Consequently determination of the equivalent volume within a factor of 2 is much more accurate than is really necessary for practical purposes. This suggests that the most convenient method of applying the new ideas to practical calculations will be to make use of a few careful determinations of the equivalent volume for various packs to estimate that of the pack under consideration. From this the F_e value required for an adequate process, according to the new ideas, can be calculated easily. If the value of n_f/C_o specified for an adequate process is $10^{-\lambda}$, D minutes is the decimal reduction time of the spores at 250° F. (121.1° C.) and v ml. is the equivalent volume, the value of F_e required for an adequate process is D $(\lambda + \log_{10} v)$ minutes. The criterion implicitly adopted in the classical methods is $F_e \not< D\lambda$ (the term $D \log v$ is omitted). Plausible values of D and λ are D equals 0.30 minute and λ equals 12 and for a No. 2½ size can of material heating by conduction v equals 13 ml. (approximately). Thus the classical methods would indicate that an F value at the center (F_e) value of 3.60 minutes was necessary whereas the newer theory would lead to a value of 3.94 min. There is no need to modify the classical methods of estimating actual F_e values for particular processing times and temperatures except in cases where they do not give accurate estimates of the contribution of the cooling phase.

Gillespy has prepared a completely new set of tables for the calculation of processes for products heating by conduction. From his tables both the F_e value and his F value for the can are easily deduced. As they stand, his tables involve the adoption of his approximation for the cooling phase but it would not be difficult to substitute another method of dealing with this part of the calculation of F_e values. The use of Gillespy's methods is clearly a satisfactory alternative to the procedure suggested in the previous paragraph.

Relative importance of factors affecting process values. It was suggested earlier that the main benefit of the recent work will probably arise from facilitating clear thinking about the relative importance of various factors, that is, in maintaining a sense of proportion. For this purpose it seems desirable to use the survival number scale. In Table 1, the effects of a number of factors on the survival number (n_f) in No. 2½ cans are estimated roughly, taking as the standard a normal cook as recommended at present. This table is an extension of Table 6 of the paper by Hicks *(5)*.

TABLE 1

Approximate effects of some factors on the survival number in a No. 2½ can processed at 240° F. (115.6° C.)

Factor	Estimate of n_f multiplied by	
	Convection heating	Conduction heating
Initial spore load under-estimated by a factor of 1000	1000	1000
Neglect of cooling phase	3	10^7
Neglect of consequences of new ideas on process evaluation	500	14
Temperature 1° F. below nominal value	3.5	3.5
Length of cook 1 minute less than nominal	20	4
Estimated decimal reduction time 10% too low	14	16
Estimated z value 10% too low	1000	1000

As already stated, the figures in this table are rough or, rather, valid only under conditions which are not completely stated. However it is clear that proper consideration of the cooling phase in solid or semi-solid packs is very important; the consequences of the new ideas are very important for liquid packs and high accuracy is desirable in the z values used in the calculations.

For different organisms the relative magnitudes of the quantities in this table might be quite different.

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Development of Off-Flavor in Shelled Peas Held in Cold Water

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Shelled Giant Stride peas held dry or submerged in water at different temperatures before blanching and freezing were organoleptically inferior to the controls processed immediately after shelling. The off-flavor was stronger in submerged samples than in the dry. Peas held at 60° F. (15.5° C.) and 50° F. (10.0° C.) had more off-flavor than those held at 40° or 33° F. (4.5° or 0.5° C.).

It is well known that delay in the processing of vined peas for freezing or canning results in the development of off-flavors *(5, 10)*, and that cooling is one method of retarding such deterioration *(9, 10)*. One obvious method of attaining rapid cooling is to suspend the peas in ice water, but prolonged holding of peas in water has been criticized as favoring off-flavor development *(1, 3, 6)*. Recent experiments at this Laboratory support this view. Inasmuch as no quantitative data appear to have been published on the subject, the results of our observations are herewith presented.

In the past at least two sources have been recognized for off-flavors in peas held in air between vining and blanching. "Delay off-flavor," as the term has been used in publications from this Laboratory, apparently results from a form of abnormal metabolism, since it has been shown that it develops rapidly only in bruised peas and that it is not due to microbial activity *(7)*. Other off-flavors, of microbial origin, may also develop *(4, 8)*. Both types of off-flavor development are retarded by cooling *(3, 4)*. In the experiments reported in this paper efforts were made to hold microbial activity to a minimum, and the off-flavors developed in submerged peas qualitatively resembled "typical delay off-flavor."

MATERIALS AND METHODS

Morse's No. 60 Giant Stride peas grown in 1949 near Milpitas, California, were used since no large-seeded freezing varieties were available. Hand picked pods were iced in the field and stored overnight at 33° F. (0.5° C.). The peas were shelled in a mechanical huller and cleaned and sized in a vibrating screen cleaner. Sizes 3-6, inclusive, were washed in running water and dried with clean towels. The control peas were processed immediately. The experimental peas, placed in covered crystallizing dishes, were either held dry on filter paper or submerged in pre-cooled water at the desired temperatures. At the end of the experiment, samples were removed for determination of pH and microbial activity. All peas, including the controls, were blanched in steam for 90 seconds at 212° F. (100° C.), fog-cooled, frozen, and stored at —10° F. (—23.4° C.).

After being cooked in half their weight of water for 5 minutes, the peas were blended to give a homogeneous sample, and vegetable dye was added to mask color differences. The resulting purees were tasted by a panel of trained judges. In addition to scoring total off-flavor, the judges indicated whether or not each sample contained "typical delay off-flavor" *(7)*. Color was scored on a portion of the cooked whole peas before blending. Total off-flavor and color were scored on a scale of 7 to 1 (Table 1). Any influence of leaching loss in submerged samples on the off-flavor scores was minimized by careful wording of the score sheet and previous training of the judges.

The tasting of the 8 experimental samples held for 9 hours and of the undelayed control peas was arranged according to a balanced incomplete block design *(2)*. The pattern of this design is such that at each judging session 4 coded samples were tasted, and by the end of the 18 judging sessions, each sample had been tasted 3 times in the presence of each of the other samples. This gave 8 replicate tastings of each sample. In addition to the coded control, 2 labeled samples, one of them identical with the coded control (unbruised, undelayed, freshly frozen material), and the other having a pronounced delay off-flavor, were also submitted each taste period, without scores, as guides to the judges.

The 4 experimental samples which had been delayed for 19 hours and one coded control were tasted together at each of an additional 5 judging sessions. One labeled unscored sample of delayed peas was presented for reference to the judges at each session.

RESULTS AND DISCUSSION

The results are summarized in Table 1. The total off-flavor scores show that in all instances the undelayed controls received a significantly higher score than any of the delayed samples (Figure 1 and Table 1).

TABLE 1

Influence of conditions of delay on pH, flavor, and color of peas

	pH		Recognition of "typical delay off-flavor" % of judgments		Total off-flavor average score [b]		Color average score [c]	
	Dry	Sub-merged	Dry	Sub-merged	Dry	Sub-merged	Dry	Sub-merged
Series I								
Controls	6.55	.....	7	....	6.5	....	5.7	....
9 hours at 33° F.	6.50	6.32	47	58	5.4	4.3	6.0	5.1
9 hours at 40° F.	6.57	6.30	45	61	5.6	4.5	6.1	5.2
9 hours at 50° F.	6.54	6.22	56	75	5.1	4.1	5.8	5.4
9 hours at 60° F.	6.44	6.17	70	81	4.7	3.5	5.8	5.5
Least significant difference at 5% level					0.5		0.4	
Series II								
Controls	6.55	.....	14	....	6.5	....	5.9	....
19 hours at 33° F.	6.76	6.51	65	71	4.1	4.0	4.9	5.2
19 hours at 40° F.	6.45	6.48	47	59	5.1	4.2	5.8	5.0
Least significant difference at 5% level					0.6		0.7	

[b] Flavor scores: 7, no off-flavor; 1, much off-flavor. Series I, 70 individual judgments per sample; Series II, 49 individual judgments per sample.

[c] Color scores: 7, bright green color; 1, gray—or yellow-green color. Series I, 40 individual judgments per sample; Series II, 26 individual judgments per sample.

Peas held submerged in water received lower scores for off-flavor than the peas held dry at the same temperature. In all but one case the lowering of the scores due to submerging was significant at the 5% level (Table 1). It is evident that more off-flavor developed in peas held under water than in those held dry (Figure 1).

There is a definite temperature effect upon the development of off-flavor in peas held either dry or

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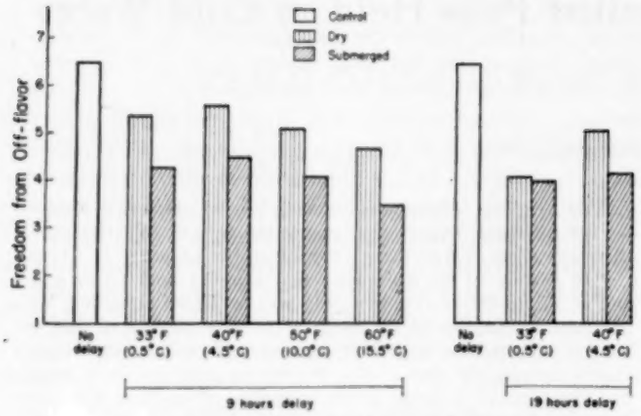

Figure 1. Off-flavor scores of frozen peas that had been held at different temperatures dry and submerged in water prior to blanching. Score 7 represents no off-flavor; score 1 represents much off-flavor.

submerged. Less off-flavor resulted from delay at the lower temperatures.

The peas delayed dry at 40° F. (4.5° C.) for 19 hours were judged to be slightly better in flavor and color than the peas held dry at 33° F. (0.5° C.) for the same length of time. A similar observation was made in a limited preliminary experiment. The differences were small, therefore more refined experiments are necessary to check this observation. Present facilities do not permit careful experiments for the establishment (or refutation) of the existence of an optimum storage temperature for shelled peas in the temperature range around 40° F. (4.5° C.).

Most of the differences in the color scores do not appear to be significant, although the trend suggests that submergence has a somewhat deleterious effect on color at the temperatures employed.

Extensive microbial activity in unblanched peas usually results in a lowering of pH and development of a sour off-flavor. The differences in pH before blanching for our experimental samples were small (Table 1). Furthermore, a count made after 21.5 hours of delay at 40° F. (4.5° C.) showed only 12,500 organisms per g. of submerged peas, where half peas and half liquid were used. Microbial contamination in these experiments was intentionally kept to a minimum by careful handling of the material and equipment. Such a low level of bacterial activity before blanching would be unlikely to influence the flavor of the frozen and cooked peas. Under commercial conditions of heavy inoculation, microbial activity for the same delay periods and temperatures would be very much greater, especially in the submerged samples, and would add to the total of off-flavors present in the frozen peas.

The exploratory experiments of 1948 with similar peas showed the same general trends as reported above but were too limited to be regarded as conclusive. However, the 1948 experiments also included bruised and unbruised peas held for 5.5 hours at 46.5° and 75° F. (8° and 24° C.) and for 18.5 hours at 33° F. (0.5° C.) both dry and submerged. These peas, together with undelayed controls, were appraised for off-flavor, color, and texture. The results showed that the unbruised submerged peas had significantly less off-flavor than the corresponding bruised peas. In fact, at the lower temperatures, the unbruised peas, delayed either dry or submerged were not significantly different from the unbruised, undelayed controls in off-flavor scores. While submergence was less damaging than bruising as a factor in the development of off-flavors, submerging appeared to accentuate the effect of bruising and delay. In commercial practice viner-shelled peas are always severely bruised.

SUMMARY

Machine-shelled peas of the Giant Stride variety were held at various temperatures, either dry or submerged under water, before being blanched and frozen. Organoleptic appraisal of cooked samples showed the following:

1. Undelayed controls, processed immediately after shelling, had significantly less off-flavor than any of the delayed samples.

2. Peas delayed under water had more off-flavor than peas held dry at the same temperature.

3. In general, the lower the temperature of holding peas the less off-flavor was produced.

4. There is suggestive evidence of qualitative similarity in the off-flavors developed in the peas held dry and those held submerged.

5. Differences in color scores, in general, were not significant at the 5% level.

6. Exploratory experiments with similar peas of the 1948 season showed that the detrimental effect of bruising and delay on flavor was accentuated by submerging peas in water for several hours.

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Evaluation of Plating Media for Citrus Concentrates

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The requirements for suitable bacteriological plating media for use with citrus concentrates are discussed and some results obtained with various plating media are reported and evaluated.

The development of frozen citrus concentrates has created many new problems, one of the most important being the bacteriological control of microorganisms that may affect adversely the quality of the canned product.

Efficient bacteriological control requires that careful supervision be constantly maintained over the manufacturing and canning operations to insure a minimum total count of microorganisms in the product, particularly those that are capable of producing an off-odor or off-flavor.

"Total count" includes not only microorganisms that are capable of growing in the product, but also those that may not germinate but still remain viable. The latter may serve as a means to evaluate clean up procedures and general sanitation and also detect the presence of organisms that may be considered potentially significant by public health authorities. It is possible that a large percentage of the organisms not capable of germinating in citrus concentrates may require a much higher pH for their optimum growth in bacteriological media than the pH of the product. It is for this reason that media covering a wide range in pH were used in these studies.

Although it was recognized that no single medium would be perfect in all respects for determining the total count of citrus concentrates, it was hoped that these studies would provide a medium which would indicate the presence of microorganisms capable of growth in the product and at the same time give a reasonable index of total contamination.

EXPERIMENTAL PROCEDURE

There were 20 cultures of bacteria and yeasts, all capable of producing an off-flavor in single strength orange juice and 20° Brix orange concentrate as determined by extensive laboratory testing and flavor panel ratings, used as test organisms.

Data regarding off-flavor production in 20° Brix orange concentrate by some strains of these organisms were reported by Murdock, Troy, and Folinazzo *(3)*. The bacterial cultures reported herein included 6 stains of the genus *Leuconostoc* and 10 strains of the genus *Lactobacillus*. One strain of *Leuconostoc mesenteroides* was obtained from Dr. Reese H. Vaughn of the University of California. Also 5 other strains isolated in our laboratory from orange concentrate, although not definitely identified as *Leuconostoc mesenteroides*, but considered to be of the *Leuconostoc* type based primarily on their gum-forming properties, their ability to ferment sucrose and pentoses, catalase reaction, and morphology were also included in the studies. The 10 strains of lactobacilli were obtained from Dr. Carl S. Pederson of the New York State Agricultural Experiment Station, Geneva, N. Y., and the 4 strains of yeasts of genus *Candida* were isolated from single strength and concentrated orange juice. The histories of the test strains are given in Table 1.

Suspensions of the test organisms were prepared by inoculating each into 25 ml. of sterile fresh California orange juice (pH 3.6, Brix 12.3) in 25 × 150 mm. screw cap test tubes. The inoculated juice was incubated 4 to 7 days at 20° C., 30° C., and 37° C., depending upon which was the most suitable growth temperature. The number of organisms present in each suspension was ascertained by making plate counts using Orange Serum Agar pH 5.4 as the plating medium.

Then 3 composite suspensions were prepared; one of 5 strains of genus *Leuconostoc*, one of 10 strains of genus *Lactobacillus*, and another of 4 strains of yeasts of genus *Candida*. In addition, a single suspension of *Leuconostoc* isolate No. 862 was also included in these studies as this was the only test organism which grew most favorably at 20° C. The suspensions forming the composites were standardized so that each consisted of approximately the same number of test organisms.

The study consisted of: 1, making plate counts of each of the composite suspensions and the single suspension referred to above; and 2, making plate counts of predetermined propor-

TABLE 1
History of strains of genera Leuconostoc, Lactobacillus, and Candida studied

Strain No.	Species	Source
	Leuconostoc	
851	*Leuconostoc mesenteroides*	Dr. Reese Vaughn, University of California
854	*Leuconostoc Sp.*	Normal frozen 42° Brix orange concentrate
856	*Leuconostoc Sp.*	Normal frozen 42° Brix orange concentrate
862	*Leuconostoc Sp.*	Off-flavored frozen 42° Brix orange concentrate
863	*Leuconostoc Sp.*	Off-flavored frozen 42° Brix orange concentrate
864	*Leuconostoc Sp.*	Off-flavored frozen 42° Brix orange concentrate
	Lactobacilli	
850	*Lactobacillus fermenti*	All lactobacilli cultures were obtained from Dr. Carl S. Pederson, of the New York State Agricultural Experiment Station, Geneva, New York
852	*Lactobacillus brevis*	
879	*Lactobacillus plantarum*	
880	*Lactobacillus fermenti*	
881	*Lactobacillus fermenti*	
882	*Lactobacillus casei*	
887	*Lactobacillus Sp.*	
888	*Lactobacillus Sp.*	
890	*Lactobacillus Sp.*	
892	*Lactobacillus fermenti*	
	Yeasts	
434	*Candida parapsilosis*	Sound oranges fallen from the trees (drops)
440	*Candida tropicalis*	Commercial juice extractor
443	*Candida guilliermondii*	62° Brix orange concentrate (normal) from evaporator
446	*Candida guilliermondii*	42° Brix orange concentrate (normal)

tionate mixtures of various concentrations of the 3 basic composites. In these studies 18 test media were used. Aliquots of the same suspension were pipetted to a sufficient number of plates and duplicate plates poured with each of the 18 media. The plates were incubated 3 to 7 days at the most favorable growth temperature for the test organisms. Counts were made daily after 48 hours. Unless otherwise stated, all tests and plates were made in duplicate. The averages of the duplicate runs were reported.

The following 18 media were investigated:

No.	Media
1	Orange Serum Agar pH 3.7.
2	Orange Serum Agar pH 5.4 (prepared from California juice).
3	Orange Serum Agar pH 5.4 (prepared from Florida juice).
4	Orange Serum Agar pH 6.5.
5	Lemon Serum Agar pH 5.2.
6	McCleskey's Agar pH 6.5.
7	Lindegren's Agar pH 5.8.
8	Potato Dextrose Agar (Bacto.) pH 5.6.
9	Potato Dextrose Agar Acidified (Bacto.) pH 3.5.
10	Dextrose Agar Acidified (Bacto.) pH 3.6
11	Tryptone Glucose Yeast Extract Agar pH 6.8.
12	Tomato Serum Liver Agar pH 6.7.
13	Tomato Juice Agar (Bacto.) pH 6.1.
14	Dextrose Tryptone Agar (Bacto.) pH 6.7.
15	Sabouraud's Dextrose Agar (Bacto.) pH 5.6.
16	Thermoacidurans Agar (Bacto.) pH 5.0.
17	Beer Wort Agar pH 6.5.
18	Molasses Agar (6° Brix) pH 5.0.

Media Nos. 1, 2, 3, and 4 were selected based on previous field studies in Florida in 1948 by Troy and Beisel *(5)*. Medium No. 5 was a modification of the orange serum agar by the substitution of lemon serum. Medium No. 6 was prepared according to McCleskey *(2)* and No. 7 according to Lindegren *(1)*. Medium No. 11 was based on the formula supplied by Dr. Carl S. Pederson *(4)*. Media Nos. 12, 17, and 18 were developed in our laboratory. Media Nos. 8, 9, 10, 13, 14, 15, and 16 were obtained from Difco Laboratories, Detroit, Michigan.

The composition of the special media used by Troy and Beisel *(5)* and those developed in this laboratory are as follows:

Orange Serum Agar (No. 1)

(A)
Tryptone	20 g.
Yeast extract	6 g.
Dextrose	10 g.
Orange serum [a]	1000 ml.

Dissolve and dispense 75 ml. in 8 oz. screw cap bottles. Sterilize 30 minutes in flowing steam (212° F.).

(B)
Bacto-agar	2.3 g.
Distilled water	75 ml.

Sterilize in autoclave 15 minutes at 15 pounds pressure (250° F.).

Mix (A) and (B) immediately before pouring of plates.

Orange Serum Agars (Nos. 2, 3, and 4)

Tryptone	10 g.
Yeast extract	3 g.
Dextrose	5 g.
K_2HPO_4	2.5 g.
Bacto-agar	20 g.
Orange serum [a]	200 ml.
Distilled water	800 ml.

The final pH of media Nos. 2 and 3 was approximately 5.4 and media No. 4 was adjusted to pH 6.5.

[a] Heat 1000 ml. of freshly extracted orange juice to approximately 200° F. and add 30 grams of Filter-Cel. Mix thoroughly. Filter through a Buchner funnel containing coarse filter paper and a layer of Filter-Cel. Discard first few ml. of filtered serum. If serum is not used immediately, it may be sterilized 30 minutes in flowing steam (212° F.) or stored unheated at 0° to —10° F. until ready for use.

Lemon Serum Agar (No. 5)

Prepared according to the formula used for Orange Serum Agars Nos. 2, 3, and 4 except 50 ml. of lemon serum was used in place of 200 ml. of orange serum.

Tomato Serum Liver Agar (No. 12)

Tryptone	10 g.
Yeast extract	5 g.
Dextrose	5 g.
Lactose	5 g.
K_2HPO_4	1 g.
Bacto-agar	20 g.
Tomato Serum [b]	100 ml.
Liver Broth Serum	100 ml.
Distilled Water	800 ml.

(No pH adjustment necessary)

Dissolve and sterilize in autoclave 15 pounds pressure (250° F.) for 15 minutes.

Beer Wort Agar (No. 17)

Sweet wort 10° balling	1000 ml.
Nutrient Agar	20 g.

Dissolve and sterilize in autoclave for 15 minutes at 15 pounds pressure (250° F.).

Molasses Agar (No. 18)

Molasses [c] 10° balling	1000 ml.
Nutrient Agar	20 g.

Dissolve, heat, filter through cotton and sterilize in autoclave for 15 minutes at 15 pounds pressure (250° F.)

The orange serum for media Nos 1, 3, and 4 was obtained from Florida Pineapple oranges 9.8° Brix, pH 3.95, and total acidity 0.84. California Valencia oranges were used for medium No. 2, producing a serum of 9.5° Brix, pH 4.0, and total acidity of 0.48.

RESULTS

A compilation of the growth of the test organisms on the various media studied is presented in Table 2. The data represents the percentage of the maximum count based on the highest count obtained by a given media at the end of the indicated incubation period. An arbitrary value of 100% growth for all media was established in each test as a basis for calculating percent of maximum count (10×10^5 for *Leuconostoc* composite, 5 strains).

Bacteria of genus Leuconostoc. The organisms included in this investigation are slime producers or "gum formers." They are coccoid in shape, gram positive, catalase negative, and form a massive formation of slime material in sucrose gelatine. They produce off-flavors in orange juice some of which have a similarity in taste to buttermilk.

A medium on which these organisms produce characteristic gum forming colonies is one of the requisites desired. Out of the 18 media investigated only 6 consistently showed this characteristic. These were the Orange Serum Agars, McCleskey's Agar, and Beer Wort Agar. However, on the Beer Wort Agar, the colonies were small and on McCleskey's Agar large characteristic colonies were obtained which coalesced and spread rapidly. This is illustrated in Figures 1, 2, and 3. On Lindegren's Agar, which has been used widely in the citrus industry as a plating medium, the colonies were very small or pinpoint and showed no gum formation, as is indicated in Figure 4.

[b] Tomato serum is prepared by filtering tomato juice through a layer of Filter-Cel.

[c] Diamalt from Standard Brands, Inc., New York, N. Y.

TABLE 2
Growth of lactic acid bacteria and yeasts on various media

Media No.	Percent of maximum count [4]						
	Leuconostoc		Lactobacillus	Yeasts	Ratio yeast to bacteria		
	Composite (5 strains)	Strain No. 862	Composite (10 strains)	Composite (10 strains)	1:3	1:1	3:1
	5 days 30° C.	7 days 20° C.	5 days 37° C.	3 days 30° C.	4 days 30° C.	4 days 30° C.	4 days 30° C.
1	51	86	9	100	29	70	76
2	72	76	98	80	100	93	100
3	76	82	100	90	100	95	92
4	59	84	92	88	81	83	97
5	100	100	96	90	100	90	100
6	43 •	76 [f]	94	91	100	83	80
7	49	80	98	86	77	84	93
8	93	80	77	100	75	89	96
9	58	78	0	88	40	73	78
10	58 •	84	0	100	40	81	74
11	61	76	100	100	92	91	100
12	100	64	99	98	94	91	88
13	68	84	71	100	83	100	86
14	96	54	67	80	71	83	93
15	27	72	90	100	73	99	92
16	100	100	100	90	96	100	88
17	40	72	90	94	91	87	94
18	64 •	2.8	83	92	....	83	82
100% growth	10×10^5	250×10^5	7.5×10^5	30×10^5	52×10^5	90×10^5	11×10^5

[4] Results based on the average of duplicate tests, except yeast composite which is the average of 4 replicates.
• Indicates one test run.
[f] 5 day count; 7 day—colonies coalesced making plate impossible to count.

The data in Table 2 indicated that Orange Serum Agars, on which characteristic colonies are produced, did not necessarily support the highest count. It should be remembered that those media which supported the largest numbers produced small colonies similar to those illustrated in Figure 4.

Based on the media investigated the 5 strain *Leuconostoc* composite required 72 to 96 hours incubation at 30° C. to obtain the maximum count. One strain No. 862 required 4 to 7 days at 20° C. to obtain the maximum count.

Bacteria of genus Lactobacillus. The strains included in this study were gram positive and catalase negative. They grew in single strength orange juice producing an acid flavor. There were 19 strains originally investigated, 10 of which were used in these studies. The most favorable growth temperature for all strains was found to be 30° C. or 30° to 37° C. Temperatures above 37° C. were not investigated. The lactobacilli strains formed very small colonies in comparison to the *Leuconostoc* organisms even after 5 days of incubation at their most favorable growth temperature. Figure 5 shows typical lactobacilli colonies (10 strain composite) on Medium No. 2 after 5 days of incubation at 37° C. Similar colonies were produced on 9 of the 18 media investigated, namely, media Nos. 2, 3, 4, 11, 12, 13, 16, 17, and 18. On the remaining 9 media the colonies were considerably smaller than those shown in the illustration. Media on which typical colonies were formed as indicated above, except for No. 13 and 18, also supported the highest counts.

For the majority of the media studied, the lactobacilli required 72 to 120 hours to produce the maximum numbers. The lactobacilli did not grow on Media Nos. 9 and 10, and only limited growth was obtained on Medium No. 1.

Yeasts of genus Candida. The yeasts grew faster than the other test organisms. Maximum counts were obtained on all test media after 48 hours of incubation at 30° C. The yeasts were not as fastidious in their growth requirements as the *Leuconostoc* and lactobacilli strains of bacteria investigated. The data in Table 2 indicated a majority of the media supported growth in the range of 90 to 100% of the maximum counts and all media investigated were in the range of 80 to 100%.

An analysis of all of the data so far presented using *Leuconostoc*, lactobacilli, and yeasts as separate composites, indicated that the highest plate counts were obtained on media Nos. 5 and 16. Medium No. 12 gave high counts but was not as favorable at 20° C. for the

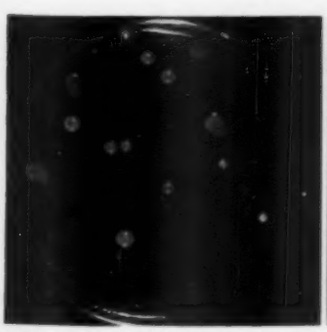

Figure 1. Characteristic Leuconostoc colonies on orange serum agar, pH 5.4.

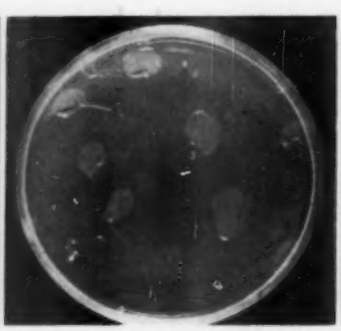

Figure 2. Leuconostoc colonies on McCleskey agar, pH 6.5.

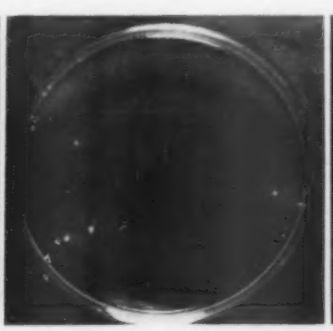

Figure 3. Leuconostoc colonies on beer wort, pH 5.5.

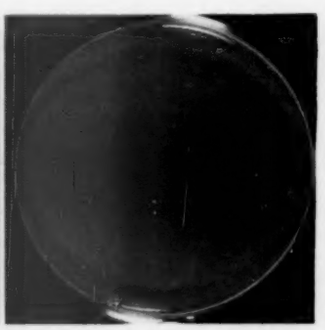

Figure 4. Leuconostoc colonies on Lindegren's agar, pH 5.8.

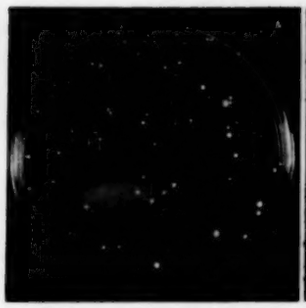

Figure 5. Lactobacilli colonies on orange serum agar, pH 5.4.

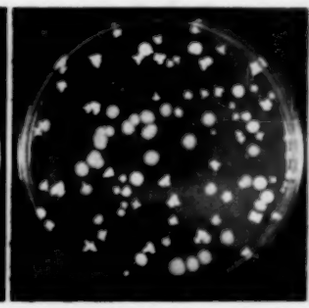

Figure 6. Yeast colonies on orange serum agar, pH 5.4.

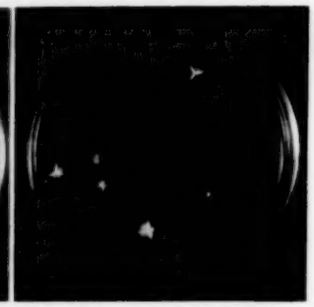

Figure 7. Yeast to bacteria ratio of 1 to 3, showing characteristic yeast, Leuconostoc and Lactobacilli colonies on orange serum agar at pH 5.4.

growth of *Leuconostoc* strain 862. Medium No. 3 which also supported high counts did not seem to be as suitable for the production of the maximum counts for the growth of the *Leuconostoc* strains investigated as for the lactobacilli and yeasts. However, Medium No. 3 was the only one of the above 4 on which gum forming colonies were formed by *Leuconostoc*. The *Leuconostoc* organisms, as the data indicated, appear to produce lower counts on this medium than on the other three media, although further replication of comparative tests might show this medium to give equally as high counts. A comparison of these media is presented in Table 3.

TABLE 3

Comparison of media supporting highest maximum count of test organisms

Medium No.	*Leuconostoc* (5 strains) % growth	Hours for maximum count	Lactobacilli (10 strains) % growth	Hours for maximum count	Yeasts (4 strains) % growth	Hours for maximum count
3	70-80	96	90-100	72-120	90-100	48
5	90-100	72	90-100	72-120	90-100	48
12	90-100	72-96	90-100	72-120	90-100	48
16	90-100	72-96	90-100	48-120	90-100	48-72

Various ratios of yeast to bacteria. The growth of *Leuconostoc*, lactobacilli, and yeasts on the various test media has been discussed. However, under commercial operating conditions the presence of a single group of these organisms would be very unlikely in citrus concentrates. A mixed flora would probably be encountered with yeasts more than likely predominating. Therefore, in order to simulate the microflora normally encountered under commercial conditions, various combinations of yeasts and bacteria were investigated. These involved the comparison of yeasts and bacteria in the ratios of 1 to 3, 1 to 1, and 3 to 1 of which the bacteria consisted of an equal percentage of *Leuconostoc* and lactobacilli as shown in Table 2.

During the course of the investigation it was observed that mixed cultures consisting of yeasts and bacteria, formed colonies faster on the test media than the composite suspensions from each individual group of organisms. Although this data is not shown it was noted as the percentage of yeasts increased in relation to the bacteria, the number of media on which the test organisms reached their maximum colony development increased. That is, for a ratio of yeasts to bacteria of 1 to 3, 1 to 1, and 3 to 1, there resulted a total of 8, 12, and 14 media respectively, supporting maximum colony development in 72 hours.

As previously indicated, media Nos. 2, 3, and 4 were the only ones out of the 18 investigated which showed the typical characteristic colony formations of yeast, lactobacilli, and *Leuconostoc* (gum forming) organisms. Of these media, Nos. 2 and 3 supported the highest counts in all combinations of yeasts to bacteria. Figure 7 shows a yeast to bacteria ratio of 1 to 3 on Medium 2 after 5 days of incubation at 30° C. It should be noted that all 3 groups of organisms were easily discernible in the photograph.

The ultimate goal of a citrus plating medium is one which could be used as a differential medium for gum formers, lactobacilli and yeasts. The medium selected should also, as nearly as possible, support the maximum number of colonies in the shortest incubation period. Out of the 18 media compared, only Media Nos. 2 and 3, satisfied this requirement. They supported the growth of *Leuconostoc*, lactobacilli, and yeasts and maximum counts were obtained, when mixed cultures of these organisms were compared. However, as indicated in Table 4, Orange Serum Agar is difficult to prepare in comparison to media in a dehydrated form.

TABLE 4

Evaluation of plating media using mixed strains of yeast, Lactobacilli, and Leuconostoc [a]

Media No.	Maximum Nos.	Rapid colony formation	Typical colony characteristics	Ease of preparation
1	—	++	—	—
2[b]	+++	+++	+++	—
3[c]	+++	+++	+++	—
4	++	+++	+++	—
5	+++	+++	+	—
6	++	+++	++	+
7	+	++	—	++
8	+	++	—	+++
9	—	—	—	+++
10	—	—	—	+++
11	+++	+++	—	++
12	++	+++	—	—
13	+	++	—	+++
14	+	+++	—	+++
15	+	+++	—	+++
16	+++	+++	—	+++
17	++	+++	++	—
18	++	+++	+	—

[a] Plating media rating: +++, good (90-100% maximum count, large characteristic colonies); ++, fair (80-90% maximum count, medium or spreading colonies); +, poor (70-80% maximum count, small colonies); and —, not recommended (less than 70% pinpoint colonies).

[b] Prepared from California orange juice.

[c] Prepared from Florida orange juice.

The composition of this serum will naturally vary with the season of the year, variety of the oranges and other factors, which may result in media of differing value from time to time. A medium which could be prepared from known constituents whose composition could always be duplicated, yet which is comparable in performance to Orange Serum Agar is the goal for further research.

SUMMARY

The growth of 6 strains of bacteria of genus *Leuconostoc*, 10 strains of bacteria of genus *Lactobacillus*, and 4 strains of yeasts of genus *Candida* on 18 media was compared as to the rate of growth, maximum counts, and colony characteristics. Composites composed of organisms belonging to the same genus and mixed cultures were compared. The *Leuconostoc* and the lactobacilli were more fastidious in their growth requirements than the yeasts. Orange Serum Agar pH 5.4, was a suitable differential medium for *Leuconostoc*, lactobacilli, and yeasts. It supported the growth of *Leuconostoc*, lactobacilli, and yeasts and resulted in maximum counts when mixed cultures of these organisms were compared.

Acknowledgment

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The Mechanics and Interpretation of Heat Penetration Tests in Canned Foods

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Heat penetration procedures and interpretation of the results obtained are discussed.

Until a few years ago, the field of process determinations for canned food products was confined to comparatively few laboratories. Now, however, many laboratories connected with the canning industry and colleges offering courses in food technology have equipment for conducting tests to establish process schedules necessary to prevent spoilage of canned foods by bacterial action. The scientific literature is not replete with references concerning the details of such tests and the interpretation of the results. Consequently, technologists have necessarily had to develop their own procedures by trial and error or learn them from others who had such experience.

The objective of this paper is to make available in one place the fundamental techniques of heat penetration measurement in canned foods and to discuss the interpretation of the data obtained. It is apparent that this and other publications cannot compensate for lack of personal experience. Technologists new to this field, however, should be materially assisted in processing work by this discussion.

The measurement of the temperatures at the slowest heating point in a can of food as it is being processed is known as heat penetration testing. In some of the earliest heat penetration studies, maximum reading thermometers were placed inside the can as reported by Kochs and Weinhausen *(15)*. The use of thermocouples for heat penetration measurement in cans in 1907 and for studying variations in temperature within a retort in 1914 are reported by Bitting and Bitting *(8)*. Extensive tests using thermocouples are described by Thompson *(25)* who is credited with the development of some of the basic equations describing the rate of heat penetration in canned foods. Bigelow, Bohart, Richardson, and Ball *(7)* described the design and use of thermocouples for heat penetration measurements and discussed many of the factors relative to process determination. A basic improvement in thermocouple design was made by Benjamin *(6)* who developed a connector type thermocouple which could be disconnected from the lead wire. Further improvement was made by Ecklund *(10)* who designed a thermocouple which does not project from the side of the test can (Figure 1). He also describes complete specialized heat penetration equipment based on this type of thermocouple. In the discussion below, it is assumed that this type of equipment is used.

THE MECHANICS OF HEAT PENETRATION TESTS

Equipment necessary for conducting heat penetration tests should include: 1. Thermocouples capable of being mounted in a can in such a manner that the hot junction is on the can axis. 2. Compensated lead wires made into a cable of several duplex leads depending on the number of cans to be tested simultaneously (no less than 6 are recommended). 3. A stuffing box so that the lead cable can be brought through the retort wall. 4. A selector switch. 5. A potentiometer for indication of the can temperatures. Only potentiometers (Wheatstone bridge type of instruments) which draw no current when in balance are suitable for this work. Direct reading millivoltmeter type meters are subject to errors resulting from variation in resistance of the lead wires. Such equipment has been described more completely by Ecklund *(10)*.

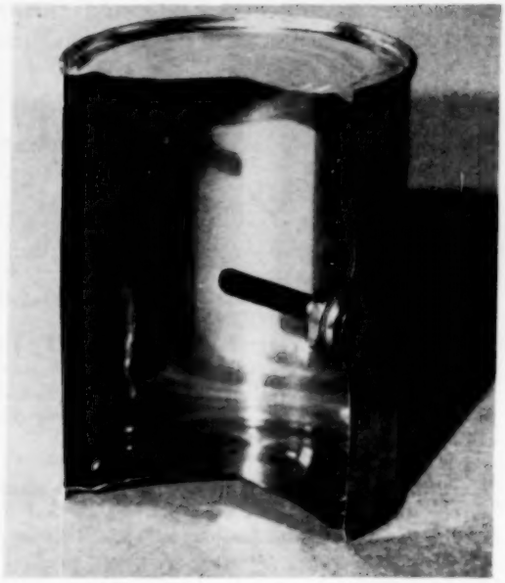

Figure 1. Non-projecting type of thermocouple installed in can.

The first step in conducting a test involves the placing of the thermocouples in the test cans. The thermocouples usually are mounted in the sides of the test cans. For products which heat by conduction (Figure 2) such as cream style corn, thickened concentrated soups, pumpkin, and most meat products, the thermocouples should be placed so that the hot junctions (located at the tips of the thermocouples) are located at the geometric centers of the cans. For products which heat by convection (Figure 2) such as clear soups and most brine packed vegetables, the thermocouple tips should be located on the can axis at a point near the bottom of the container. For No. 2 cans the point is about ¾ inch (19 mm.) above the bottom and for No. 10 cans, about 1½ inches (38 mm.) above the bottom when the cans are processed in an upright position. These points have been shown by Jackson and Olson *(14)* to be the slowest heating points for these 2 types of products. Between these 2 extremes are products which exhibit the so-called broken curve heating, examples of which are vacuum packed corn, brine packed spinach, syrup packed sweet potatoes, and tamales. Products which fall in this

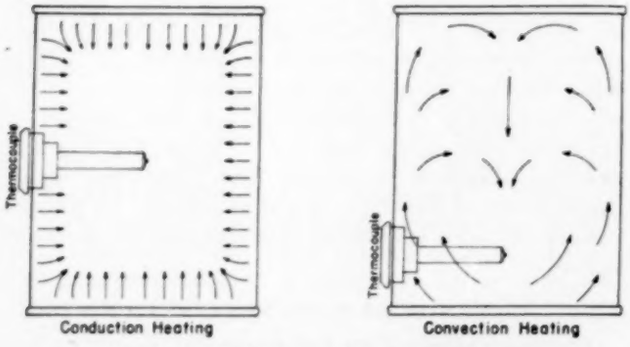

Figure 2. Mechanism of heat penetration.

class may have the slowest heating point either at the geometric center of the can or near the bottom on the can axis. Test cans in an initial heat penetration test should have thermocouples located in both positions to determine which point is the slower heating.

For round cans the thermocouple is preferably mounted in the body or side. If the thermocouple is mounted on the can end, flexing of the end during processing may shift the junction to such an extent in some products as to give erroneous heating data. With some of the taller rectangular cans as, for instance, the 6 lb. luncheon meat container, the flexing during process takes place in the side panels and in such cases it is advisable to mount the thermocouple in the can end. With smaller rectangular cans the thermocouple can be mounted either on the end or on the narrow side. In any event, if the product heats by conduction, the hot junction should be located at the geometric center.

Since lead cables are generally made with either 7 or 10 wires, usually from 6 to 9 cans are used for making each heat penetration test, the remaining lead being used to measure the temperature of the heating medium. In regard to the number of tests necessary, it is well to continue testing until the technologist is satisfied that he has obtained heating data that approach the slowest heating rate that might be obtained in commercial operation. For extremely rapid (convection) and extremely slow (conduction) heating products, only a few heat penetration cans are usually required because there will be little variation in the heating rate from batch to batch and can to can. Special care should be taken with products which exhibit broken curve heating because a slight change in packing procedure may result in a wide variation in heating rate. Figure 3 illustrates the diverse

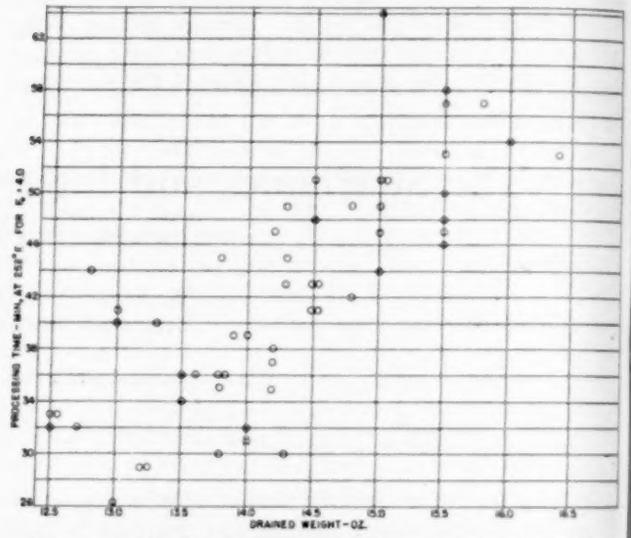

Figure 3. Results of heat penetration measurements in No. 2 cans of mustard and turnip greens.

results obtained in heat penetration tests on mustard and turnip greens. Tamales and most other products packed asparagus style will vary in heating rate depending on the tightness of the pack and the position of the can (horizontal or vertical) in the retort. The heating rate of vacuum packed products may vary with the fill-in-weight or can vacuum. Thus in preparing heat penetration cans it is frequently advisable to include both normal and overfilled cans and to include any other variables which might influence the rate of heating.

Filling of fluid products usually can be carried out on commercial equipment with the thermocouples mounted in place on the cans. If the product is made up of large pieces it is better to fill the test cans by hand so that the thermocouple tips may be imbedded in a large piece of the product and also to prevent thermocouple breakage. For tuberous vegetables, such as brine packed potatoes, it is not considered necessary that the center of the food particle receive the full sterilizing heat since this area is essentially sterile. Consequently, the thermocouple tips are usually imbedded in such solid food particles to a depth of only ¼ to ½ inch (6.3 to 12.7 mm.).

With meat products which are stuffed into the cans, the thermocouple may be removed during the stuffing operation, the thermocouple receptacle only being mounted on the can. This will prevent breakage of the thermocouple during the stuffing operation. After the can is filled, the thermocouple can be inserted and tightened and the can closed in the usual manner.

When convenient, probable or actual commercial techniques should be followed in exhausting and closing the test cans. Variations in can vacuum, closing technique, can position during processing, etc., which may differ from normal, should be studied in case such variations may affect the rate of heating.

In brine packed products and conduction heating products in round cans, the degree of vacuum in the cans has little effect on the rate of heating and the commercial exhausting and closing procedure need not be strictly followed. Vacuum packed vegetables *(27)*, and semi-solid products packed in rectangular or other noncylindrical shaped cans may vary in heating rate as a result of variations in closing vacuum. These cans should be vacuum closed in the commercial manner although it is advisable to include low vacuum cans as a variable in order to evaluate the effect of this condition.

The test cans should be handled so that the initial temperature is in the range that will be obtained in commercial practice. Although correction for variations in initial temperature can be made in process calculations, it is good technique to keep these corrections to a minimum.

Alstrand and Benjamin *(1)* showed that a distorted picture of the heating rate may be obtained if the temperatures within a test can are non-uniform. This can occur when cans of a slow heating product are filled hot and allowed to stand for some time before being processed. Under these conditions the product near the surface of the can would be cooler than that at the can center. If the heat penetration factors are derived from data based on tests in cans having an initial can center temperature greater than the average temperature, the lag factor obtained will be higher than the true value for this product. The converse of this can be true for a slow heating product filled cool and subjected to a thermal exhaust. To minimize these errors, the industry has accepted the definition of initial temperature as the average temperature of the product at the start of the process.

In convection heating products, natural convection will prevent any appreciable difference in initial temperature in different parts of the can. In semi-fluid products, uniform temperature distribution usually can be obtained by shaking the cans just before the start of the process. In heavy consistency or solid products which are filled hot, the best practice is to start the process with a minimum amount of delay after filling and closing the cans.

It is the practice in some laboratories to locate the test cans in various parts of a retort along with the commercial containers. This cannot be considered good practice since it is, in effect, measuring 2 variables at one time. If slow heating is obtained in one or more cans it is not possible to say whether this resulted from slow heat penetration into the product or from poor heat distribution in the retort. Thus, unless the temperature distribution in the retort is known positively to be good, the data obtained may not be the true heating rate of the product. If the temperature distribution is good, such practice is unnecessary. If it is desired to check the temperature distribution in a retort, this can be done by locating exposed thermocouples in various parts of a loaded retort.

The basic rate of heat penetration is not affected by the number of cans in the retort. In making a heat penetration test, therefore, the test cans may be placed in a group in an otherwise empty retort (Figure 4) or in a group in a loaded retort. The position of the cans should be the same as that which will be used in commercial practice, that is, either vertical or horizontal. The test cans should be numbered to correspond with the number of the lead wires so that examination data for each can may be recorded later along with the proper heating data.

One lead wire should be reserved for measuring retort temperature. A thermocouple should be attached to this lead and the thermocouple located in the vicinity of the test cans. The temperature indicated by this thermocouple should be used as the reference temperature in all calculations because it is subject to the same lead wire and instrument errors as the thermocouples in the cans. These errors, therefore, are automatically compensated. Frequently the retort thermocouple temperature does not exactly check the retort thermometer reading which may or may not be accurate.

After all thermocouples are connected to the heat penetration cable an initial temperature reading of each can should be made and recorded.

In determining the heating factors for a product, it is standard practice to correct the heating curve for "zero come-up time." This is done because the come-up time of the retort, that is, the time required to vent the retort and bring it to processing temperature, is a variable factor and unless its effects are eliminated from the calculations, a factor for come-up time would have to be recorded along with the heating factors for the product. In order to minimize the retort come-up correction, the retort should, except in specified instances, be brought to processing temperature as quickly as possible. Obviously the error in correcting for a 3 minute come-up is insignificant as compared with the possible error for a 20 minute come-up time.

Figure 4. Cans located in retort ready for heat penetration test.

The retort should, of course, be operated in accordance with established and accepted standards as regards venting to remove the air during come-up and bleeding to prevent the accumulation of non-condensible gases during the process. Recommended procedures have been published by the National Canners Association *(16, 17, 22)*. The retort must be carefully controlled at processing temperature throughout the entire processing period.

Timing of the heat penetration test is started when the steam is turned on. The initial temperature readings for all the test cans should be made just prior to turning on the steam. The "come-up" time, or the time required for the retort to reach processing temperature as indicated by the retort thermocouple, should be noted and recorded. The time interval between successive temperature readings for each thermocouple will vary with the heating rate of the product. The readings may be taken as frequently as every minute for a rapid heating product and, in some instances, as infrequently as every 20 minutes or more for large cans of a conduction heating product. Enough points should be recorded to insure that an accurate curve can be constructed.

If the temperature readings obtained for 1 or 2 cans appear to be inconsistent compared with the other cans in the test, it is desirable to continue the test until all thermocouples read within 1 degree of retort temperature as a check on the accuracy of the equipment. If a thermocouple fails to indicate a temperature within 1 degree of retort temperature (as indicated by the retort thermocouple) after prolonged heating, some error in the thermocouple or lead wire is indicated and the heating results for that can should be discounted.

If the graphical method of Bigelow, Bohart, Richardson, and Ball *(7)* is to be used for process determination or evaluation, processing should be stopped and cooling started at the end of the estimated processing time. Cooling data will be required for this method of evaluation and is obtained by continuing to record temperature readings during the cooling period until the can temperatures are less than 200° F. (93° C.). The "water on" time and the time the test cans are submerged should be recorded. This later time can be determined by a rapid fall in the temperature of the retort thermocouple.

When cooling data are obtained for pressure cooled cans of a conduction heating product, a record should be made of the retort pressure during the cooling period. The retort pressure during the cool in such instances has been shown to have a marked effect on the rate of cooling *(12)*. The correlation be-

tween cooling rate of conduction heating products and retort pressure is limited to cans, which are flexible containers. Some comparative heat penetration studies in cans and jars were reported by Townsend et al. *(26)*.

The heat penetration test is not considered complete until the test cans have been examined. This examination is usually made on the day following the test to allow time for solids in the can to re-absorb some of the moisture lost during the process. The cans should be examined for all factors which might affect the rate of heating. These include can vacuum, net weight, drained weight, and thermocouple location (as in a piece of meat). Occasionally such factors as consistency, viscosity, total solids, and pH will be of interest.

The heating data from the slowest heating can shown to be satisfactory from the examination are used as a basis for calculating the process time or sterilizing value as the case may be. Average data are not used since it is the spoilage organisms in the slowest heating container that must be destroyed. It is for this reason that the technologist should continue to conduct tests until he is assured that the slowest data obtained are representative of the slowest heating cans in commercial production. On this same premise it is not advisable to base a process on 1 or 2 test cans included in the commercial batch, particularly if the product is erratic as regards rate of heat penetration, since there is no assurance that the test can or cans chosen will be representative of the cans which have the slowest rate of heating. It is more satisfactory to establish a process time on the basis of exhaustive heating studies and standardize on this process unless changes in the formulation or packing procedure are made. In such a case the heat penetration studies should be repeated.

INTERPRETATION OF HEAT PENETRATION TESTS

The objective of heat penetration determinations is, of course, to obtain data to be used in process calculations. The 3 methods which are commonly used in analyzing the data are usually referred to as the graphical method, the formula method, and the nomogram method. These methods are described by Bigelow, Bohart, Richardson, and Ball *(7)*, Ball *(3, 4)*, and Olson and Stevens *(18)*, respectively. These 3 methods are summarized in a recent publication *(2)*. A combined formula and graphical method has been described by Ball *(4)* who also has shown how process calculations can be applied to pasteurization problems *(5)*.

Other methods of analysis have been described by Stumbo *(23, 24)* Hicks *(13)*, and Gillespy *(11)*. All of these workers have approached this problem from the premise that there is probability of survival of spoilage bacteria throughout the can contents and not just at the can center. While the development of these methods will be followed with great interest by students of processing technology, there is but little likelihood that greatly different results will be obtained by application of the new methods as compared with the methods in common use.

Both time and temperature are essential elements in the sterilization of canned foods. Sterilization can not be achieved simply by the attainment of a certain temperature in the can center. For every temperature there is a time element that must be satisfied to destroy bacteria. Using the methods developed by Ball, the amount of lethal heat at the slowest heating point in the can is totaled and is expressed as the sterilizing value (F_o) of the process. The F_o value has been defined as the equivalent number of minutes at 250° F. (121.1° C.) when no time is required to heat to 250° F. or cool to sublethal temperatures and when the thermal death time curve slope (z) is equal to 18° F. The z value is the temperature difference between 2 points on the thermal death time curve which differ by a multiple of 10 on the time scale. Although this value is subject to some variation, an average value of 18° F. has worked out well in practice.

From the heat penetration data, it is possible to evaluate the amount of sterilizing heat supplied by a specific process for the product tested. Conversely, it is possible to start with a specific sterilizing value and calculate a processing time at a given retort temperature. It is apparent, therefore, that the process time and sterilizing value are dependent variables, one must be known in order to calculate the other. To calculate a process, it would appear necessary to know the thermal resistance of spoilage organisms contained in the product and, indeed, it would be desirable. Although frequently it is known what organisms are likely to cause spoilage in a particular product, the degree of contamination or the resistance of the particular strain that will be present at any particular time cannot be foretold with accuracy. Thermal death time tests are sometimes used to aid in establishing satisfactory sterilizing values. Small glass tubes or small cans of the product involved are inoculated with spores of a resistant organism, sealed, subjected to various processes and incubated or subcultured to determine the thermal resistance of the organism in the product for which a process is to be determined.

More often, however, established processes which have given satisfactory results for many years are used as yardsticks in selecting sterilizing values to be used in process calculations for new products. As an example, calculated sterilizing values for some commercial processes are shown in Table 1.

As indicated in the table, the same sterilizing value is not always used for both large and small cans of the same product. The tendency is to use higher sterilizing values for the larger can sizes of convection heating products and lower sterilizing values for larger can sizes of conduction heating products. The processes for No. 10 cream style corn were established in actual practice at the lowest level which would give reasonable freedom

TABLE 1

Calculated sterilizing values (F_o) for some current commercial processes

Product	Can sizes	Approximate calculated sterilizing value (F_o)
Asparagus	All	2 to 4
Green beans, brine packed	No. 2	3.5
Green beans, brine packed	No. 10	6
Chicken, boned	All	6 to 8
Corn, whole kernel, brine packed	No. 2	9
Corn, whole kernel, brine packed	No. 10	15
Cream style corn	No. 2	5 to 6
Cream style corn	No. 10	2.3[a]
Dog food	No. 2	12
Dog food	No. 10	6
Mackerel in brine	301 × 411	2.9 to 3.6
Meat loaf	No. 2	6
Peas, brine packed	No. 2	7
Peas, brine packed	No. 10	11
Sausage, Vienna, in brine	Various	5
Chili con carne	Various	6

[a] The actual sterilizing value of No. 10 cream style corn cooled under pressure may be twice as high as the calculated value, as shown by Hemler, Alstrand, Ecklund, and Benjamin *(12)*. The calculated F_o values for conduction heating products are based on the slope of the cooling curve the same as the heating curve with a constant lag factor. Packing or processing conditions which vary the cooling curve slope are not taken into account in the formula method.

from spoilage. This was necessary in order to maintain acceptable quality in the large can sizes. As would be expected, therefore, the sterilizing values of standard processes for large cans of cream style corn and other conduction heating products are usually a little lower than for smaller can sizes of the same products. As noted above, the actual sterilizing value for No. 10 cans of cream style corn evaluated by the graphical method *(7)* cooled under pressure may be much higher than the calculated sterilizing value according to Ball *(3)* and actually approaches the sterilizing value obtained for standard processes in the No. 2 size can.

Heat penetration tests and inoculation studies by Sognefest and Benjamin *(20)* and Roberts *(19)* confirm the need for higher sterilizing values in larger cans of convection heating products. Processing studies on No. 2 and No. 10 canned peas indicated the need for 22 to 44% higher sterilizing value for the latter. Similar studies of brine packed whole kernel corn, although somewhat less reliable because of the variable rate of heating of the product, also indicated the need of a somewhat higher sterilizing value for the larger can size. The necessity for higher sterilizing values for large cans of convection heating products may be partially explained by the fact that the larger cans contain several times more spores than smaller containers. Because of liquid circulation in the product, all organisms receive substantially equal heat treatment. If both can sizes received equivalent sterilizing values, more probability of spoilage would exist for the cans containing the larger number of spores.

Sognefest, Hays, Wheaton, and Benjamin *(21)* reported extensive thermal death time can studies on the effect of pH on the thermal process requirements for vegetable purees. In this work it has been noted that the carbohydrate content of the food as well as the pH has a marked effect on the thermal resistance of test organism P.A. 3679. Based on the correlation found between pH, carbohydrate content and thermal resistance, the figures in Table 2 may be useful as a guide for the selection of sterilizing values:

Although no meat products were included in the tests on which the above table was based, the values do not appear to be out of line for meat products.

In the foregoing discussion, the calculation of processes is based on the heating data from the size can involved in the heat penetration test. The technologist starting out in this work will be interested in tables for converting heat penetration factors from one can size to another. These have been prepared by Olson and Stevens *(18)*. These tables work satisfactorily for most products but in converting from very small cans to large containers or vice versa, caution must be taken. In such instances it is preferable to conduct heating tests in both can sizes.

TABLE 2

Proposed sterilizing values (F_s) in relation to pH and carbohydrate content of canned foods

pH of product before heating	Percent carbohydrate in canned vegetable products[b]		
	3-6	9-12	15 and above
4.5 to 5.0	0.5	1.0	2.0
5.0 to 6.0	3.0	4.5	6.0
6.0 and above	4.0	6.0	8.0

[b] The 3-6% range includes all products in groups 1 and 2, the 9-12% range includes all products in groups 3 and 4, and the 15% and above includes all products in groups 5, 6 and "miscellaneous high" as classified by Chatfield and Adams *(9)*.

SUMMARY

The experimental technique involved in establishing processing schedules for canned foods has been described. The factors likely to be most important in influencing the rate of heat penetration are discussed.

The analysis of heat penetration data is described along with applicable thermal death time data. Although the thermal death time data may be obtained from laboratory experiments, the usual practice is to calculate the necessary factors by application of heat penetration data to processes known to be satisfactory.

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Color Measurement as a Quality Control Tool[a]

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Food colors are measured for two purposes: first, to serve as an index of appearance to the customer, and second, to serve as an index of some other quality factor such as the ripeness of a fruit. When color is to be used as an index of appearance, it is important that the particle size distribution, degree of packing, wetness of the product, and other factors which affect appearance be the same at the time of measurement as they will be when the consumer sees the product. On the other hand, when color is to be used as an index of some other quality factor it is important that the particle size distribution, wetness, and packing be adjusted to have minimum effect on the readings regardless of their effect on the appearance of the product.

In food processing, color is important in at least two different respects. First, color affects the appeal of the product to the consumer, since it is an important appearance factor. Second, experience has taught that color is often directly related to some other aspect of quality; for example, redness as an index of ripeness in tomatoes; the development of a brown color as flavor develops in baking bread, roasting coffee, toasting cereals; and conversely, the absence of brownish or yellow off-colors as a quality index in evaporated milk and coconut. Therefore, evaluation of color, usually by visual methods, has long been standard practice in many food industries. Comparisons are made with standard samples of the food products or with color handbooks, such as the Munsell system *(12)*, or the Maerz and Paul "Dictionary of Color" *(11)*.

The food industry, in seeking optimum quality and uniformity of product, increasingly feels the need for instrumental methods to give a quantitative, objective, and reproducible measure of appearance. In developing color measurement for quality control of any product, it is most important to define the purpose of the measurement.

The definition of purpose controls the manner in which the sample is prepared for measurement. When color is to be used as an index of appearance to the consumer, it is important that the other factors affecting appearance be allowed to influence the color reading. That is, the particle size distribution, degree of packing, moisture content, and other factors must be the same at the time of measurement as they will be when the consumer sees the product. On the other hand, when color is to be used as an index of quality, it is important that particle size, degree of packing, moisture content, etc., be adjusted to standard conditions before the measurement is made.

Some illustrations may help to explain these distinctions. Recently Worthington, Cain, and Wiegand *(15)*, Kramer *(8)*, and Younkin *(16)* have developed methods for directly measuring the reflectance of tomato juice or tomato paste. These methods are indices of the appearance to the consumer. They also give an index of a quality factor, ripeness. On the other hand, Kramer and Guyer *(9)* and Davis *(2)* earlier extracted the pigment from tomato paste and measured the transmission of the extract in a single region of the spectrum. This gives only an index of ripeness.

Having defined the purpose of the method, the next step is to determine the spectral characteristics of the product to be measured. This is best done by obtaining spectrophotometric curves of a number of samples which in some way show a perceptible difference to the eye. If your laboratory does not own a spectrophotometer, this step of the work can be sent out to a testing laboratory. The resulting spectrophotometric curves are then studied to find the nature of the differences between the samples and the kind of measurement which can be made. The curves may differ in shape, having a more or less definite peak in a shifting region of the spectrum (Figure 1). These samples would vary in hue.

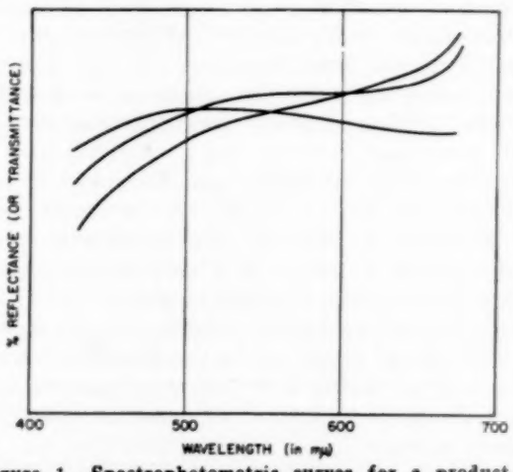

Figure 1. Spectrophotometric curves for a product which varies predominantly in hue. No one measurement can define the relationship of one curve to the group of curves.

For example, tomatoes may be more yellow or more red, depending on variety, maturity, etc. On the other hand, the curves for all samples may have similar shapes but may differ only in the intensity of the color in part or all of the spectrum (Figure 2). This was the case of tapioca starch as reported by Holleman *(5)*.

For the first situation (Figure 1), where hue varies and the curves differ in shape, the quality control method will require a measurement of color as a whole. This physical difference may be measured wavelength by wavelength using a spectrophotometer or it may be measured using a colorimeter with a series of filters, each passing light from a portion of the spectrum. One set of 3 filters, the tristimulus filters, is designed to approximate the response of the human eye *(6)*.

For such measurements, the instruments recommended by Kramer *(8)* in his tomato paste work are useful. In one of these instruments, the Hunter Color Difference Meter, the electrical

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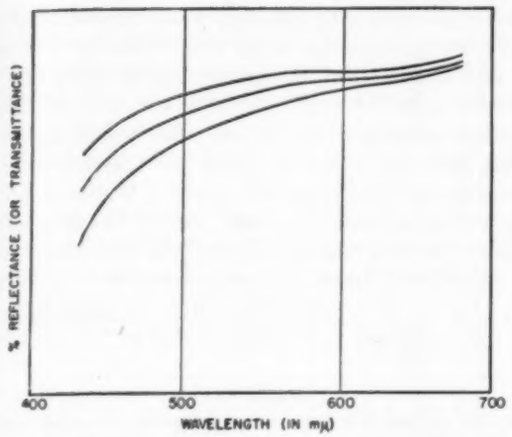

Figure 2. Spectrophotometric curves for a product which varies predominantly in intensity. One measurement can define the relationship of one curve to the group of curves.

outputs from tristimulus filter-photocell combinations are put through a computer circuit and emerge as numbers which are values of total reflectance, yellowness to blueness, and greenness to redness. A refinement of this instrument, specifically designed for use on cotton, automatically plots the indices of yellowness to blueness and total reflectance on a graph when cotton is placed on the instrument *(13)*.

The other instrument used by Kramer was the Photovolt Reflection Meter. This consists of a control box containing the indicating galvanometer and electrical adjustment, and a portable "search unit" containing the light source and photocell. The search unit is placed directly on the surface of the sample to be measured. The light beam passes through a filter to the surface of the sample. Direct mirror-type reflectance is not measured, but diffuse reflectance at an average angle of 45° is picked up by the measuring photocell. Several interchangeable filters are available, so that any of a number of spectral bands may be isolated. The values describing the hue of the sample, which are automatically computed by the Hunter instrument, are in this case calculated from readings with several of these filters.

For the second situation, where intensity varies and the reflectance curves have similar shapes, it is possible to select a portion of the spectrum where differences between samples are large and to make measurements only in this region, using a single light-filter-photocell combination. This single reading serves as an index of intensity variation between samples. Most of our work has fallen in this category. For this type of measurement we have found that simpler photometers such as the Photovolt Reflection Meter are suitable. Since the variation is described by one measurement, the impulse from the meter can be used to operate recorders and controllers in much the same manner as with a thermocouple impulse. With proper precautions against stray light and undue surface variation, the sensing units of such photometers have been placed above a conveyor belt and continuous measurements made.

Preparation of the sample for the color measurement may require considerable ingenuity. To get a reproducible reading, the surface viewed by the instrument must be fairly uniform and must be representative of the sample. For a uniform dry powder or a clear liquid, it is simple to pack or pour the sample into a glass container for measurement. However, it has already been indicated that a measurement which is to be an index of appearance to the consumer requires that no change be made in particle size, moisture content, or other factors contributing to appearance. When the particles are large or non-uniform in shape or in color, the instrument must be made to view them in such a way as to even out the non-uniformities. One such method is suggested by Thompson *(14)* who placed a number of swatches of laundered cloth on a phonograph turntable and revolved them under the viewing port of the instrument.

Cloudy liquids provide another problem since it is rarely possible to count on a uniform turbidity from one sample to another. For a quality index filtering or centrifuging may be satisfactory to clear the solution although some color may be absorbed on the filter paper. Worthington, Cain, and Wiegand *(15)* pointed out, however, that the particles in fruit juice were a part of the appearance to the consumer, and that filtration could not be used if an appearance measurement was desired. They therefore measured the reflectance of the juice instead of the transmittance. Grading of maple syrup is done visually by comparison with permanent glass color standards developed by Brice and Turner *(1)*. Their comparator box includes clear and cloudy blanks to be used with the glass standards depending on the clarity of the samples. Gillett, Meads, and Holven *(4)* have compensated for turbidity in transmittance measurements on white sugar solutions. They measure the transmittance measurements on white sugar solutions. They measure the transmittance of blue light, which is affected by both color and turbidity. Then the same solution is measured with an infra-red filter which gives a reading for turbidity alone. The difference between the 2 readings represents the color of the liquid phase.

For quality index measurements, more leeway in sample preparation is possible. Samples may be ground to obtain better sampling and to create smaller, uniform particles. Moisture may be added to a dry material to form a paste as in the flour method of Kent-Jones and Martin *(7)*. Gillett and Holven *(3)* used pressure in preparing brown sugar for reflectance measurements. They found that too little pressure left the surface lumpy, whereas too much pressure caused separation of syrup from the crystals with a resultant glassiness on the surface. They controlled the pressure by placing a uniform weight or volume of brown sugar in a metal ring and compressing it to a given extent with a plunger. The resulting pellet had a uniform surface for measurement.

The foregoing examples should give an idea of the type of manipulation which can be used in preparing samples for color determinations. Let us stress once more that the most important phase of any color measurement method is the preparation of a reproducible sample for measurement.

Tentative methods may be evaluated for reproducibility by making a series of measurements on a single production sample and statistically analyzing for the standard deviation. The magnitude of the standard deviation is compared with the range of readings by the same method on limits of production. From these 2 pieces of information, the number of detectable shades within the production range can be calculated. We have developed and used methods which would detect as many as 50, or as few as 3 distinct shades.

An important feature of any color measurement is the standard by which the instrument is calibrated before each measurement is made. Actually, the reflectometers and colorimeters are comparators. They compare the sample to a known standard which is usually in the form of a porcelain-enameled metal or ceramic tile for reflectance, or a liquid solution for transmittance measurements. It is important that these standards be as spectrally similar to the product being measured as possible for although minor spectral changes in emission of the light source or sensitivity of the photoelectric cell may take place, they will have little or no effect upon the value obtained from such a comparison *(10)*.

Let us illustrate these principles with some examples from our own experience. The first product was to be evaluated for appearance to the consumer. It is sold as a fine powder. Since we wanted an index of its appearance to the consumer, we did not alter its appearance by grinding or screening or packing it under pressure. Since the powder contains a wide range of particle sizes, it tends to segregate easily and therefore it is necessary to mix the sample carefully. The standard deviation of readings on the samples taken directly as they come from the container was 2.41. The standard deviation of readings on samples from well-mixed containers was 0.25, one tenth as much.

Figure 3 shows the reflectance curves for samples of this product. Curves for the transmittance of the

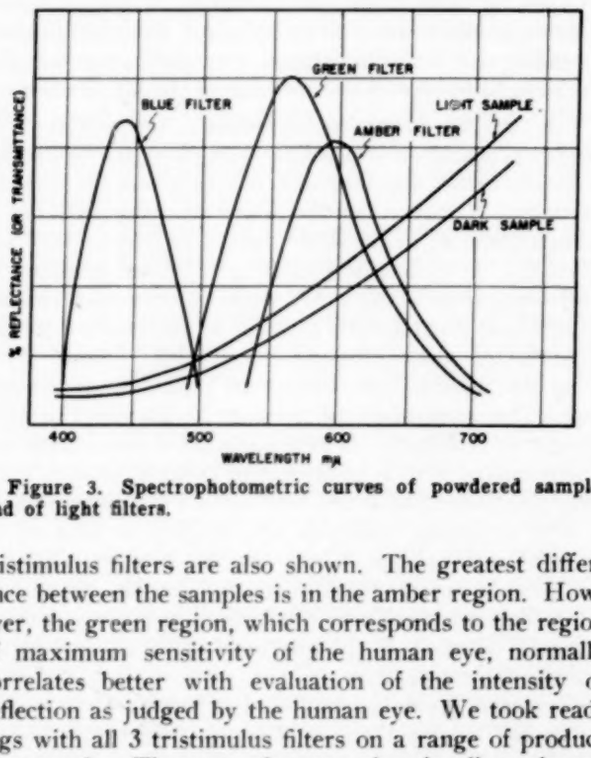

Figure 3. Spectrophotometric curves of powdered samples and of light filters.

tristimulus filters are also shown. The greatest difference between the samples is in the amber region. However, the green region, which corresponds to the region of maximum sensitivity of the human eye, normally correlates better with evaluation of the intensity of reflection as judged by the human eye. We took readings with all 3 tristimulus filters on a range of production samples. These samples were also visually evaluated by laboratory personnel. The correlation coefficients between visual ranking and instrument readings were −0.98 for the green filter and −0.96 for the amber filter over the normal range of plant production. Since the tristimulus green filter gave a slightly higher correlation coefficient and since in general it should correlate better with visual evaluation, it was chosen for this product. The limits of confidence for this method were ±0.5%, the production range was 10 units. The sensitivity of this method was therefore quite adequate.

Another product will illustrate the use of color to control processing. In this case, the visual evaluation of color intensity has long been used as an index for completion of cooking operation. When cool, the product is crisp and is ground before being evaluated. Hue does not vary appreciably and the tristimulus amber filter was chosen more or less arbitrarily to give us an index of color intensity. The effect of particle size on color measurement is illustrated under "a" in Table 1.

TABLE 1
Effect of particle size and preparation on reflectance measurements

	Average reflectance	Standard deviation
a. Effect of screen size		
Through 28 mesh, on 35 mesh	8.0	1.17
Through 35 mesh, on 60 mesh	31.5	0.67
Through 60 mesh, on pan	38.75	0.50
b. Effect of type grinder		
Grinder A	8.6	1.02
Grinder B	13.6	2.04

Note that both the value of the readings and the reproducibility of the results are affected by the particle size. The value of the readings is affected because the greater surface on the fine particles reflects more light (just as the color of copper sulfate becomes lighter as it is ground finer). We believe the effect on reproducibility is a result of the better sampling and smoother surface obtained with the large number of finer particles.

This method also illustrates admirably the effect of the type of grinder on the reflectance of the product. Table 1 "b" shows the average readings and standard deviations obtained on samples from 2 grinders. The difference in average readings was doubtless due to the type of fracture produced by the different grinders. We believe the difference in standard deviations was a result of the design which permitted re-setting grinder A much more precisely than we could re-set grinder B.

One more principle was illustrated clearly in the development of this method. The product is reddish in cast as indicated in the reflectance curve in Figure 4. Convenience dictated at first that we use a gray standard indicated in the same figure. However, when measurements were made on the same samples with a number of different instruments, variations of about 4 or 5 units were found and traced to differences in the photocells. When we changed to a standard having reflectance

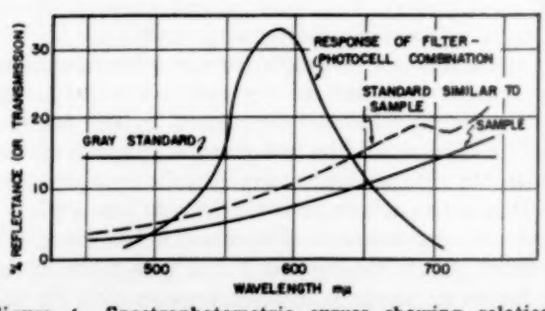

Figure 4. Spectrophotometric curves showing relation of sample to standards.

characteristics similar to the sample (within the range of the filter-photocell combination), differences in readings between instruments were reduced to within 2 units.

In developing the method for another product, the objective was to replace a tedious subjective method by a simple objective method. There were apparent differences in hue, ranging from blue to yellow, in the incoming raw material which is normally white. However, the reflectance curves on a representative group of samples, shown in Figure 5, showed that all the samples were yellow (lacking reflectance of blue light) compared to magnesium oxide. It therefore seemed reasonable to assume that we could use one filter as an index of the color of these samples. Since the greatest differences were in the range of the blue filter, this one was used. The correlation between the instrument readings with the blue filter and the acceptability of the samples as indicated by the experts is shown in "A" in Figure 6.

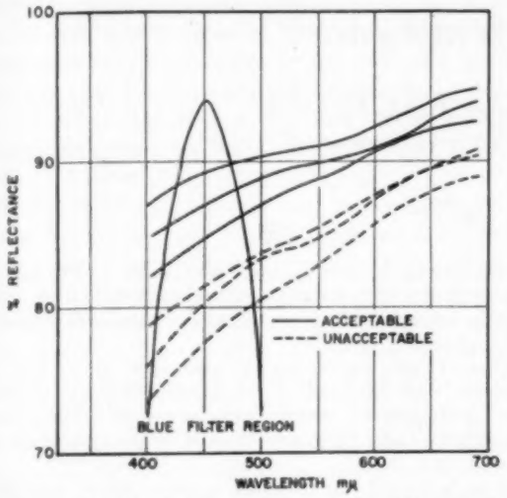

Figure 5. Spectrophotometric curves of white samples varying mainly in intensity of blue reflectance.

This method offers an excellent opportunity to illustrate a case where tristimulus colorimetry had a definite disadvantage. Yellowness is customarily measured, particularly in the paper industry, by the yellowness factor, the difference between the amber and blue readings divided by the green reading. Yellowness factors were determined for these samples and were plotted as indicated in Figure 6. It is at once apparent that the yellowness factor is not as good an index as the blue reflectance alone of the property of the material that the experts evaluate. The limits of confidence in both the blue reading and the yellowness factor are also indicated on the chart. Note that the ratio of the limits of confidence to the range of acceptability is much better for the single measurement by the blue filter than for the calculated yellowness factor. The yellowness factor is computed from the difference between two values, the amber and blue readings, each having a small error. When these absolute errors are applied to the relatively small differences between the readings, the overall error expressed as a percent is greatly magnified.

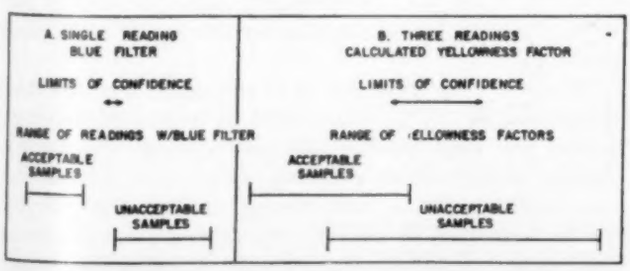

Figure 6. Schematic comparison of single measurement and tristimulus measurement.

Another product also had apparent differences in both hue and intensity. Several sample curves are given in Figure 7. In this product it is possible to adjust the intensity of color independently. Experience has shown that adjusting the intensity of color in this product is not adequate since there are still differences in the appearance when color intensities in the region of the green filter are identical. Tristimulus colorimetry is therefore indicated in this case.

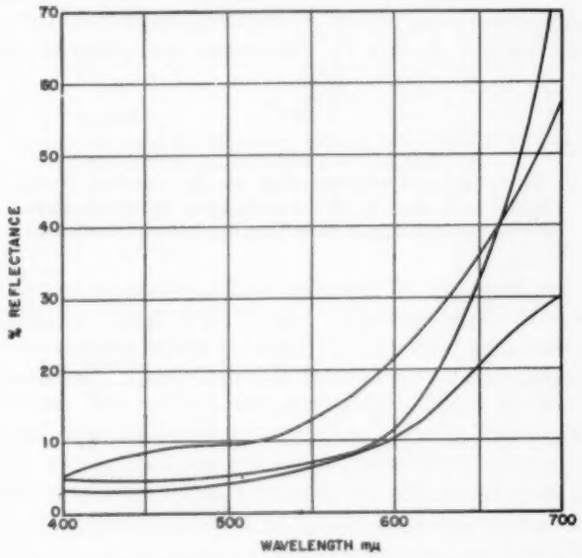

Figure 7. Spectrophotometric curves for samples differing in both hue and intensity.

CONCLUSIONS

Although the development of a method of measuring color presents an individual problem with each food product, the underlying principles outlined in this paper can be applied to almost all types of food products. It is up to the ingenuity of the food technologist to develop the method, following these principles, which will serve as a simple and yet a most valuable tool in food processing.

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Ascorbic Acid and Meat Color[a]

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The effects of ascorbic acid on the color of fresh ground pork and on the development of cured meat color with nitrite have been investigated and discussed.

The addition of ascorbic acid to meat may result either in improvement or loss of meat color. Coleman, Steffen, and Hopkins *(3)* found that the application of ascorbic acid to the surface of small cuts of fresh meat served to retain or improve the desired red color of refrigerated meats. Ascorbic acid has also been found to protect cured meats from surface fading *(1)*. On the other hand, the addition of ascorbic acid to ground meat preserved by freezing resulted in a dulling of the surface *(7)*.

The reactions of hemoglobin with ascorbic acid have been reviewed by the authors in a previous paper *(9)*. The red pigment of meat is mainly myoglobin which has been much less thoroughly studied than hemoglobin. However, it is known that myoglobin, like hemoglobin, is readily oxidized to the ferric form *(6)*, also that in the presence of ascorbic acid a green cholemyoglobin, similar to that from hemoglobin, is formed *(5)*. The red pigment from cured meat is a nitric oxide myoglobin. It would seem, therefore, that observations on the effect of ascorbic acid on hemoglobin might be expected to carry over to myoglobin and possibly to meat. The present study on meat, patterned after the previous report on hemoglobin, was undertaken to determine the effect of ascorbic acid on color retention in fresh pork as well as its effect on color fixation in nitrited pork.

METHODS AND MATERIALS

All experiments were carried out on fresh ground pork obtained from a local packing house. The pH of all samples ranged from 6.0 to 6.4. Color changes were noted visually, since no objective method for following such changes in meats was available.

When ascorbic acid was added it was prepared in concentrated solutions, adjusted to the pH of the meat, before addition to the meat. When ascorbic acid and nitrite were both used they were added together in the same concentrated solution. The pH of the ascorbic acid was adjusted before addition of the nitrite, since at pH values below 5.0, nitrite is rapidly decomposed with visible evolution of gas.

Because of rapid bacteriological spoilage at 45° C. and 24° C, experiments were confined to refrigerated, frozen, and cooked samples. Refrigerated samples were stored in beakers covered with aluminum foil. They were cooked in the same beakers set in boiling water.

Meat to be frozen was prepared in 50-g. patties, separated by squares of wax paper, packaged in pliofilm bags fastened with a rubber band, protected by outer cardboard cartons and stored at 0° F. (—17° C.). Changes in color of the meat could be observed through the pliofilm without opening the bag. Patties were removed at intervals for odor and flavor observations and peroxide determinations on the fat.

Fat extraction and peroxide determinations have been described previously *(8)*.

RESULTS

Experiments in the absence of nitrite. The addition of ascorbic acid to refrigerated ground meat resulted generally in a brightening of the surface as compared to controls without ascorbic acid, after standing for some days in the refrigerator. The extent of improvement varied with different samples of meat. It was noticeable with 0.02% ascorbic acid but more marked with higher concentrations (0.1% and 0.5%). The higher concentrations, especially 0.5%, brought about irregular greying in underlying areas, even though the surface was improved. The oxidation of hemoglobin brought about by ascorbic acid as well as the normal rate of metHb formation in the absence of ascorbic acid are known to be more rapid at low oxygen pressures (page 477, ref. 6). No greening was observed in any of the samples during refrigerator storage or subsequent cooking.

Ascorbic acid was tried in frozen meat only at a concentration of 0.1%. As would be predicted from its action on hemoglobin solutions during freezing, it usually brought about a dulling of the color. The dulling was probably due, as with hemoglobin solutions, largely to the formation of metHb. No greenish discolorations were observed and the dulling was reversible upon thawing; that is, frozen patties containing ascorbic acid, when

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exposed to air and thawed, became brighter at the surface than controls without the ascorbic acid.

Salt had the same effect on meat as on hemoglobin solutions; that is, acceleration of the browning or greying. This was particularly marked in the freezer, where greying of the meat containing salt, either with or without ascorbic acid, proceeded very rapidly. Salted samples also developed unpleasant odors and rancidity of the fat much earlier than control samples. These effects of salt on frozen pork have been noted frequently in the literature *(4, 8)* and have been ascribed to an acceleration by salt of the hemoglobin catalyzed oxidation of unsaturated fats. However, salt did not specifically affect the reaction between hemoglobin and unsaturated fat *(2)*. It would appear that salt has an independent accelerating effect on the oxidation of hemoglobin and on the oxidation of unsaturated fats.

The usefulness of ascorbic acid in preserving the color of fresh meats without nitrite would seem to be limited to the surface application of small concentrations (0.02 to 0.1%). Under these conditions brightening of the surface, or longer retention of the original bright red color, may result in refrigerated but not in frozen meats.

Color fixation in nitrited meat samples. As with hemoglobin solutions *(9)* neither nitrates nor dextrose had an appreciable effect on meat color in the experiments reported here and were therefore omitted after preliminary trials. All of these experiments were carried out with 0.1% ascorbic acid and 0.02% nitrite, adjusted to the pH of the meat and added together. Immediately upon mixing the meat turned grey, due presumably to metHb formation by the nitrite.

Nitric oxide color fixation in such meats was irregular and incomplete when the meats were stored in the refrigerator. In the absence of ascorbic acid, the development of cured meat color was negligible. In the presence of ascorbic acid, color usually developed within a few days in underlying areas.

Color development could be obtained with samples containing ascorbic acid by heating for approximately 10 minutes at a temperature of 70° C., or immediately upon heating to higher temperatures. Heating nitrited samples without ascorbic acid usually resulted in spotty and incomplete color development. On subsequent refrigerator storage of cooked samples, those containing ascorbic acid retained their color for several weeks, or until spoilage occurred, whereas those without ascorbic acid faded rapidly at all surfaces exposed to air.

It was also noted that samples cooked with ascorbic acid were much superior in odor and flavor to controls cooked without the ascorbic acid. The latter developed an unpleasant, stale odor during storage which increased in intensity. The odor was not that typical of rancidity; peroxide development was negligible. Samples cooked with ascorbic acid alone were definitely superior in odor to the control samples and those cooked with ascorbic acid and nitrite were still better, after both refrigerator and freezer storage.

Color fixation could be obtained by freezing as well as by heating. Excellent color development was obtained in 1 to 4 days storage at —17° C. in meat containing ascorbic acid and nitrite.

Salt definitely improved color fixation of nitrited samples under all conditions tried. Frequently the addition of salt resulted in complete color fixation under conditions where only slight or irregular color fixation could be obtained without the salt. Color development could be obtained by heating meat containing nitrite and 2% salt, even without ascorbic acid. Color could also be developed in the refrigerator in nitrited meat samples containing both salt and ascorbic acid. In frozen nitrited meat samples containing salt and ascorbic acid, color development was very rapid and intense. Even in the absence of ascorbic acid, color developed slowly over a period of several months in frozen nitrited samples containing salt, but the color faded very rapidly on thawing.

Table 1 summarizes results of an experiment on frozen pork sausage which illustrates several of the points discussed above. The fresh ground pork used for the experiment was thoroughly mixed and divided into 8 portions with additions to each portion as indicated in the table. The pink color noted in 4 and 8 was fully developed when the first observations were made after 4 days. The less uniform pink color in 7 developed gradually over a period of 4 to 6 weeks. All of the salted samples had developed fat peroxides, but off odors and flavors were detected only in 5 and 6. Samples 7 and 8, especially the latter, had a definite cured meat flavor as well as the typical cured meat color after cooking.

TABLE 1

Effect of ascorbic acid and salt on color and rancidity of frozen ground pork stored for 2 months at —17° C.

Additions to ground pork	Peroxide value	Surface appearance
1. Control, no addition	0	Slightly dulled
2. H_2A, 0.1%	0	Increased dulling
3. Nitrite, 0.02%	0	Brownish grey
4. H_2A + nitrite	0	Bright pink
5. Salt, 2%	11.5	Grey
6. Salt + H_2A	13.3	Grey
7. Salt + nitrite	8.1	Pink
8. Salt + H_2A + nitrite	9.0	Very bright pink

Ascorbic acid slightly accelerated the development of rancidity in this experiment. From other work on artificial systems *(10)*, it might be expected to have a variable effect on rancidity in meat, sometimes accelerating and sometimes inhibiting, depending upon the tocopherol content and fatty acid make up of the fat. Combinations of ascorbic acid with small amounts of ethylene diamine tetraacetic acid invariably gave protection in lard-hemoglobin mixtures, but the combination has not been tried on frozen meats.

In a single experiment in which the pH of meat containing nitrite and ascorbic acid was varied from 6.2 to 5.4 (by addition of lactic acid) and the meat subsequently frozen, the samples at the lower pH had greater color fixation after 24 hours in the freezer, but the differences were not great and all samples looked much alike (bright pink) after 3 days in the freezer. Evidently good cured meat color may be developed in a few days in the freezer even at the upper limits of the pH range of normal meat and in the absence of salt.

It should be emphasized here that the experiments reported were mainly concerned with the rapidity and

completeness of color development, not with stability of the color and subsequent fading under various meat storage conditions. Rate of color development and subsequent color stability are not necessarily correlated.

SUMMARY AND CONCLUSIONS

The effects of ascorbic acid on the color of fresh ground pork and on the development of cured meat color with nitrite have been investigated.

Ascorbic acid may be useful in preserving the red color at the surface of refrigerated (but not frozen) meats. It is generally useful in developing and maintaining cured meat color in meats containing nitrite. Uniform cured meat color in ground pork may be achieved by the incorporation of a mixture of nitrite and ascorbic acid, followed by heating or freezing to accelerate color fixation. Salt and low pH values accelerate the development of the cured color.

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Book Review

The Lipids, Their Chemistry and Biochemistry, Volume I, Chemistry. By Harry J. Deuel, Jr. 982 pages, Interscience Publishers, Inc., New York, N. Y., 1951. Price $18.50.

For the first time there has been brought together in readily available and compact form a great mass of up-to-date data and information on the chemistry of the lipids, classified broadly to include the glyceride fats, waxes, phospholipids, cerebrosides, fatty acids, aliphatic and cyclic alcohols, aliphatic and cyclic hydrocarbons, carotenoid and other pigments, and the fat-soluble vitamins which are associated in natural fats and waxes. Each of these classes of lipids has been the subject of one or more monographs in the past, but Dr. Deuel has packed into a single volume most of the important facts concerning all of them.

The book is divided into ten chapters, the first and shortest of which is devoted to the general characteristics and classification of the lipids. Each of the other nine chapters is concerned with one class of lipids beginning with the fatty acids and glycerol (156 pages). Chapter III (139 pages) covers the chemistry and properties of the neutral fats; Chapter IV (100 pages) is concerned with the waxes, higher alcohols including sterols, triterpenes, glyceryl ethers, colored fats and hydrocarbons. An equal amount of space is devoted to the phosphatides and cerebrosides (Chapter V), which is followed by an excellent account of the carotenoids and related compounds (154 pages). Chapter VII is reserved for the chemistry and properties of the A group of vitamins (71 pages). These two chapters reflect the close association and collaboration of the author with Prof. Zechmeister so generously acknowledged in the Preface. Chapters VIII to X (120 pages) are devoted to the chemistry and properties of the D, E, and K groups of vitamins, respectively.

In addition to 203 tables, 64 figures, and 5 plates, the author has made generous use of formulas and equations to depict the structures and transformations of the important lipids. In addition to an author and comprehensive subject index, the volume contains an index of generic plant and animal sources from which the principal lipids are derived. The corresponding common names are given in the main subject index. More than 3,500 carefully verified references are cited in connection with the text.

Attention should again be called to the erroneous representation of the stearic acid crystal (Fig. 18), one-half or the other of which should be rotated through an angle of 180°. This incorrect figure, originally contained in a pre-war German work, has been reproduced in at least four recent American works, including one by the present reviewer.

Despite this and a few minor slips of quoting earlier instead of more recent work and views with respect to some of the minor components of natural fats, the book is highly recommended as an invaluable tool for research workers, teachers, students, and all others engaged in or generally interested in the broad field of chemistry and biochemistry of the lipids.

The author deserves much commendation for the preparation of this work and the publishers should be congratulated on the excellence of the typography, paper, and binding. K. S. Markley.

Factors Influencing the Quality of Tomato Products. I. Surface-Localized Pectic Enzymes Inactivated by Blanching

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Pectic enzymes in ripe Pearson and San Marzano variety tomatoes occur in greatest quantity near the surface of the fruit; therefore a large proportion of such enzyme activity may be destroyed prior to crushing by passing whole fruit through a steam or hot water blanch. Such treatment can improve the retention of pectic substances during processing and tomato products of higher consistency may be obtained.

It is well known that enzymic destruction of pectic substances during processing is one of the factors contributing to low consistency in tomato products *(2, 6, 7)*. This destruction can be greatly reduced by rapidly heating freshly crushed tomato macerate to 185° F. (85° C.). However, the action of these enzymes is very rapid *(4)* and it has been found in the course of earlier work in this laboratory *(6)* that 100% retention of pectic substances is not obtained even under the best practical commercial conditions of rapidly preheating freshly crushed tomato macerate. This is due to the fact that heating and inactivation of pectic enzymes cannot be made instantaneous and therefore a finite time interval exists during which some destruction of pectic substances will occur. Increased retention of pectic substances, with resulting improvement of consistency in tomato products, might be possible if some practical means were found to inactivate a large percentage of the pectic enzymes prior to crushing and preheating.

British workers have reported *(8)* that oxidizing enzymes in the tomato fruit are located largely in the skin and outer portions. If pectic enzymes are similarly located, they might be inactivated by scalding in steam or hot water prior to crushing. This heat treatment would probably reduce the degradation of pectic substances in the short interval between crushing and attaining the proper preheating temperature of 185° F. (85° C.)

This paper reports investigations of the location of pectic enzymes in ripe tomato fruit and application of an improved processing method whereby greater retention of pectic substances is attained.

EXPERIMENTAL

Location of pectic enzymes. The tomato fruit has been described *(1)* as consisting of an outer wall (pericarp) the radial walls, the core, and the locular cavities containing the seeds embedded in a jelly-like parenchyma tissue originating from the placenta. The pericarp, in turn, is described as consisting of 3 distinct sections: the epidermis, a single layer of cutinized cells; the epicarp, consisting of 3 or 4 layers of collenchyma cells; and the mesocarp, consisting of parenchyma tissue which constitutes the bulk of the pericarp.

Whole ripe tomato fruit (Pearson var.) was dissected to separate these morphological sections. The fruit was cut in halves through the axis. One half was then separated as completely as possible into epidermis, epicarp, mesocarp, radial walls and core, and locular cavity contents. The weight of each was determined and the individual fractions were macerated in a micro-blender with an equal weight of 10% sodium chloride solution,[b] and filtered. Pectin-methylesterase (PM) and pectic acid-depolymerase (DP) were then determined in each extract *(6)*. The remaining half of the fruit was comminuted with an equal weight of 10% sodium chloride solution and PM and DP activities were determined on filtered extracts. The total pectic enzyme content of the whole fruit was calculated from these data and used in calculating the percentages of the total pectic enzyme activity of the fruit in each of the sections. In this manner, 25 ripe Pearson tomatoes were analyzed. The percentage distribution of pectic enzymes in the sections was averaged for all fruits. The data are presented in a condensed form in Table 1 together with the approximate thicknesses of the sections involved. In the same manner, 25 ripe San Marzano (pear-shape) tomatoes were analyzed with essentially the same results as given in Table 1, except that the thicknesses of morphological sections differed.

TABLE 1

Location of pectic enzymes in ripe Pearson tomatoes

Section of fruit	Approximate thickness of sections	Percent of total PM activity found in section[c]	Percent of total DP activity found in section
Epidermis plus epicarp	1/16 inch	25-50	25-50
Total pericarp including epidermis and epicarp	¼-½ inch	65	65
Radial walls and core	.....	35	35
Locular cavity	.....	0	0

[c] The tomatoes examined varied in absolute pectin-methylesterase units (PMU) content from 15 to 35 PMU per g. on a whole fruit basis. Although there is considerable variation, the total pericarp makes up approximately 45% of the weight of the whole fruit; the radial walls and core about 35%; and the locular cavity contents about 20%.

Discussion

In Table 1, the data for the epidermis and epicarp have been combined because of the uncertainty of perfect separation of the fractions by dissection and the high variability among individual fruits in the ratio of PM and DP in the 2 sections. For the same reasons no value is given for the mesocarp. This value was replaced by a value for the total pericarp, which includes epidermis, epicarp, and mesocarp. It is interesting to note that, in spite of variations in the percentage of pectic enzyme contents of these 3 fractions, the epicarp was always found to contain a higher percentage than either the epidermis or the mesocarp. This fact strongly indicates the localization of pectic enzymes near the fruit surface. It is also interesting to note that the distribution of the two enzymes was always related to differences in color and distribution of color. Thus the redder tomatoes were found to have a higher total pectic enzyme content than more pale tomatoes. In redder

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[b] In contrast to experience with citrus fruit *(3)*, it was found that the above method of extraction applied to all types of tomato tissue yields the same amount of PM as extractions employing combinations of salt and elevated pH.